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DEVELOPMENT OF THE PISTILLATE FLOWER AND STRUCTURE OF THE FRUIT OF TUNG (ALEURITES FORDII)[1]

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INTRODUCTION

The study of the pistillate flower and developing fruit of the tung tree (*Aleurites fordii* Hemsl.) reported herein was undertaken to determine the general morphological characteristics of the fruit. Although this tree is the commercial source of tung oil, there are many undescribed details concerning the development and structure of its fruits and the seeds from which the oil is extracted. Investigation of the internal structure of the fruit is of particular importance as a basis for further studies of the origin of the oil and the nature of its elaboration. Furthermore, the terminology of the various botanical structures of the fruit needs to be clarified.

The tung industry is as yet too young to have developed definite horticultural varieties. Efforts are being made by the Bureau of Plant Industry, the State experiment stations, and a few private growers to develop varieties by selection, but as yet commercial orchards consist almost entirely of seedlings, and each tree differs from the others in one or more characters. Since seedling tung trees are of different genotypes, there is great variability in shoot growth, flower-bud initiation, development of the embryo, and growth and maturity of the fruit, and these characters are not so constant as they are in horticultural clones of other orchard crops.

REVIEW OF LITERATURE

The mode of flowering and fruiting in the tung tree has been described by Dickey and Reuther (*8*).[2] Several variations in the flowering and fruiting habits are mentioned.

Abbott (*1*) found that under Florida conditions in 1926 and 1927 initiation of fruit buds in tung occurred between May 10 and October 1, the majority of the buds differentiating during the latter half of June. He believed that the early blossom-bud differentiation of May 10, 1927, was hastened materially by a shortage of available soil moisture.

According to Croizat (*6*), some systematists believe that the Euphorbiaceae are closely related to the Juglandaceae. A number of studies have been made on the fruits of *Carya* and *Juglans* species; these are of interest, since in some ways these fruits are similar to those of tung. Crane and Hardy (*5*) reported two periods in the de-

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[2] Italic numbers in parentheses refer to Literature Cited, p. 377.

velopment of the pecan fruit: (1) The period of growth in size, which begins at blossoming and probably ends with the hardening of the shell, at which time the nuts have attained full size; and (2) the period of filling of the nut, which begins probably about the time the shell begins to harden and ends with the separation of the nut from the shuck. These two periods in the development of pecans were analyzed further by Thor and Smith (*20*), who reported that during September under central Texas conditions there may be a critical period in the filling of the nuts due to unfavorable environmental conditions, since nuts are largely filled during that month.

In a series of papers J. G. Woodroof (*25*, *26*), Woodroof and Woodroof (*27*, *28*), and N. C. Woodroof (*29*) reported on the time of initiation of flower buds and the development of the pecan nuts to maturity. Staminate flowers were initiated during May and pistillate buds after the beginning of growth in the spring. It was found that the internal character of the staminate buds does not change from the time of harvest in November until the middle of February. The hull of the pecan fruit is described as a modified calyx, which is shed at maturity. The shell is the ovarian wall; it begins to harden about 4½ months after pollination and requires about 3 weeks for completion of hardening. All space within the ovary not occupied by the middle septum and the enlarging ovule is filled with parenchymatous tissue of the endocarp. The nucellus with the small embryo sac fills the integument in the early stages. The seed coat is a development of the single integument inside of which the embryo develops. Beginning about 9 weeks after pollination, the nucellus is gradually absorbed, and the embryo eventually fills the entire seed coat.

Adriance (*2*) pointed out that the pistillate flower of the pecan contains an orthotropous ovule surrounded by a single integument, that the shell consists of two carpels, that the four-valved husk develops from the lower parts of the calyx lobes, and that the flowers are sessile and are borne in clusters of two to six.

Shuhart (*18*) termed the hull of the pecan a "specialized stem," the shell a "hard lignified exocarp," the ridges on the inner surface of the shell "mesocarp," and the so-called packing tissue "parenchymatous endocarp." He classified the pecan as a kind of pome fruit that consists of a specialized stem, the husk, which surrounds a normally two-carpelled ovary containing a single ovule.

Langdon (*11*) described and compared the embryogeny of *Carya* and *Juglans*. The so-called packing tissue of the genus *Carya* was described as follows: "During early fruit development, all space within the ovary not occupied by the middle or primary septum is filled with the light parenchymatous tissue of the endocarp, which takes its origin from the inner tissue of the carpel walls and from the septum."

Later, Langdon (*12*) described the walnut ovary as a fleshy, urn-shaped tube, the product of conjoint growth of the stem and carpels. The involucre was described as a cupular structure of fleshy axis, which encloses the ovary and is united with it, forming the fibrous fleshy indehiscent husk.

The morphological development of *Juglans regia* L. was reported by Nast (*16*). The kernel of the nut is the embryo, the greater part

of the husk of the matured fruit is involucre, a thin layer of the husk is perianth, and the shell is the greater part of the ovary wall. The vascular system has three cycles: An outer cycle ending in the involucre; an inner, perianth-carpel cycle; and an innermost ventral cycle.

METHODS AND MATERIALS

Terminal tung buds were collected at weekly intervals from December 1938 to September 1940 from 50 trees selected to represent varied growing conditions and different genetic types. From 3 to 5 buds were collected at random from each tree each week. Buds were killed in various fixatives, including formalin-aceto-alcohol and 1 percent picric acid in 50 percent alcohol, and Navaschin's solution. Tissue was dehydrated in the ethyl-butyl-alcohol series, embedded in paraffin, cut 8μ to 15μ thick, and stained in 1 percent aqueous crystal violet or in some cases in iron-alum haematoxylin. As a check on the paraffin technique, buds collected on the same dates were dissected under a dissecting microscope and photographed by means of microtessar lenses of 32-, 48-, and 72-mm. equivalent focus.

Young fruits collected at the time of and subsequent to fertilization were fixed in formalin-aceto-alcohol and a modified Navaschin's fluid containing twice the usual amount of formalin. The same techniques were applied as in the case of the buds.

The fruits cannot be sectioned in the conventional manner when collected at the time of or subsequent to the hardening of the seed coat, which occurs about 3 months after bloom. Such fruits were cut with a heavy butcher knife into cross and longitudinal sections about 10 to 15 mm. thick, fixed in formalin-aceto-alcohol, dehydrated, and cleared in glycerin and refined pine oil. Sections were photographed by transmitted light to show vascular structure.

The tung fruit consists normally of five seeds or ripened ovules enclosed in a fleshy hull, but the number may vary from three to more than five. In this paper the term "seed" will be adhered to, although it is common and accepted terminology to speak of the seeds as "nuts."

OBSERVATIONAL DATA

TERMINAL GROWTH

The overwintering terminal buds are almost invariably mixed buds, and in addition to several vegetative growing points they contain a varying number of differentiated flowers. Usually within 2 weeks after the flowers have set fruit, new shoots start from the vegetative growing points. The number of such new shoots produced on each terminal varies considerably. Eight terminals of the previous season observed on each of fifteen 7-year-old trees produced an average of 4.7 shoots, with an average length of 30 cm. In a few cases as many as 17 new shoots have been observed on 1 terminal (*10*).

TYPES OF INFLORESCENCE

Tung trees are commonly monoecious and predominantly synoecious, with an average pistillate-staminate ratio of about 1 : 27 (table 1). However, it is not uncommon to find trees that produce inflorescences that are preponderantly staminate throughout the

entire tree, such as tree No. 18, table 1, with a ratio of 1 : 440. These trees are known by the tung growers as "male" trees. It is also possible to find trees that have very low ratios, such as tree No. 25 with a ratio of 1 : 5. These trees are often characterized by aberrant, often abortive, staminate flowers and are commonly designated as

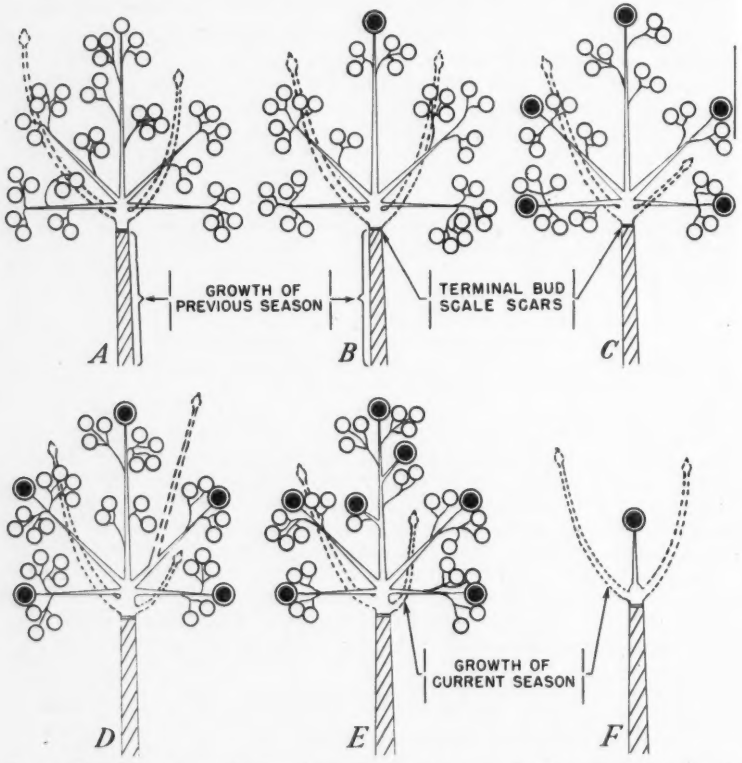


FIGURE 1.—Diagrammatic drawings of several types of inflorescence commonly found on tung trees: *A*, Type found on staminate trees; *B*, type found on trees bearing fruit as "singles"; *C*, type found on trees bearing fruit in a "bunch cluster"; *D*, same as *C*, except for a terminal shoot appearing as a lateral branch from a flower stalk; *E*, type found on trees bearing fruit in a "grape cluster"; and *F*, type found on trees bearing only one functioning pistillate flower in an inflorescence and from none to several usually abnormal staminate flowers. Open circles indicate position of staminate flowers in the inflorescence; circles with black centers indicate position of pistillate flowers; and dotted lines indicate the position and points of origin of potential terminal shoots.

"female" trees. Still other trees show tendencies toward producing perfect flowers in certain inflorescences.

Flowers are produced in cymose inflorescences from the pulvinate growing point of the terminal bud. The number of main flower stalks or rachises arising from the growing point of one terminal bud varies from one to many. Figure 1 shows diagrams of several types

of inflorescence based on the position of the pistillate flower or flowers. Although the majority of the terminals of any one tree tend to produce only one of the types of inflorescence illustrated, it is not uncommon to find several types on one tree.

TABLE 1.—*Pistillate and staminate flower ratios computed from counts made in April 1941 on 10 inflorescences from each of twenty-eight 6-year-old trees selected at random in two different locations*

Tree No.	Pistillate flowers	Staminate flowers	Ratio of pistillate to staminate flowers	Tree No.	Pistillate flowers	Staminate flowers	Ratio of pistillate to staminate flowers
	Number	*Number*			*Number*	*Number*	
1	24	638	1:27	16	19	688	1:36
2	16	167	1:10	17	19	513	1:27
3	14	326	1:23	18	3	1321	1:440
4	18	321	1:18	19	9	414	1:46
5	14	251	1:18	20	14	162	1:12
6	19	516	1:27	21	10	179	1:18
7	5	243	1:49	22	24	198	1:8
8	7	250	1:36	23	13	111	1:9
9	4	422	1:105	24	12	266	1:22
10	9	139	1:15	25	34	186	1:5
11	8	252	1:32	26	10	160	1:16
12	7	414	1:59	27	11	308	1:28
13	9	225	1:25	28	4	245	1:61
14	8	325	1:41				
15	8	359	1:45	Average			1:27

Figure 1, *A*, shows staminate inflorescence consisting of five flower stalks, all of which bear staminate flowers. The terminus of each rachis may be branched dichotomously or trichotomously. Lateral flower stalks, also branched dichotomously or trichotomously, may occur on the main flower stalks. Figure 1, *B*, shows the inflorescence found on trees that bear their fruit as "singles," that is, one fruit per terminal bud. In this type, from one to many flower stalks occur, of which only the centermost is terminated by a pistillate flower. The so-called bunch cluster is illustrated in figure 1, *C*, which shows that each of two or more flower stalks is terminated by a pistillate flower. The remaining flowers of the inflorescence are staminate. The "grape cluster" or "true cluster" (fig. 1, *E*) differs from the "bunch cluster" in that pistillate flowers occur on branches from one or more of the main flower stalks. Usually the centermost rachis is the only main flower stalk to produce pistillate flowers both at the terminus and as laterals. The pistillate type of inflorescence (fig. 1, *F*) is characterized by having only one main rachis, which is terminated by a single pistillate flower. This type may have from none to several staminate flowers that are usually abnormal.

The shoots arise from the terminal growing point immediately below the points of origin of the flower stalks except in certain cases such as that illustrated in figure 1, *D*. In this type, the shoot arises from a lateral bud on the flower stalk.

FRUIT BUD FORMATION

The term "fruit bud," rather than "blossom bud," is used here because this study is primarily concerned with the pistillate flower and its role in fruit development.

In 1940 (*15*) at Bogalusa, La., tung started shoot growth about the second week of April. Terminal buds collected and examined at weekly intervals showed the first evidence of flower-bud initiation during the second week in June (pl. 1, *A*). Initiation of pistillate flowers was found to occur until late October. In 1939 the first evidence of pistillate flower initiation was observed during the last week of May, and initiation continued throughout the summer until late October. Primordia in the individual pistillate flowers develop in acropetal fashion (pl. 1, *B*) and appear in the following order: Sepals, petals, staminodes, and carpels. However, the development of the entire inflorescence within the terminal bud is basipetal. The central and terminal flower develops first, and the lateral flowers, usually staminate develop later.

For convenience in recording development, the terminal buds examined were classed in four empirical groups:

(1) Preinitiation stage.—No evidence of initiation of flowers.

(2) Predeterminate stage.—Flower-bud initiation as evidenced by the widening and flattening of the meristematic axis and the appearance of primordia, which eventually form a two- or three-parted valvate calyx (pl. 1, *A*).

(3) Determinate stage.—Pistillate distinguishable from staminate flowers (pl. 1, *B*). Staminate flowers (pl. 1, *D*) produce stamen primordia in an ascending spiral over the entire face of the central meristematic zone, while the pistillate flowers produce the petal and staminodal primordia in very close peripheral spirals. These protuberances soon form two concentric cycles of primordia at the periphery of the central meristem, which then becomes dome-shaped. The carpel primordia appear later at the periphery of the dome-shaped meristem (pl. 1, *C*).

(4) Advanced stage.—Carpel primordia have developed and the young pistil has the appearance of a five-lobed and scalloped cup (pl. 1, *E*). The concavity of the cup is divided by partitions into locules, which are on the same radii as the convex lobes of the carpels.

RESTING BUDS

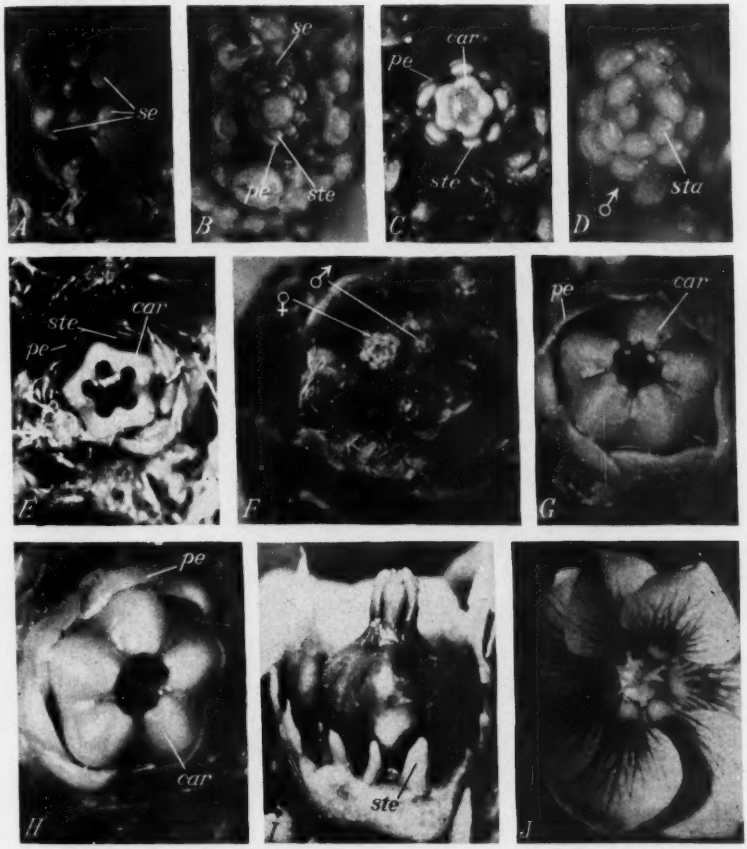
When the developing pistillate bud reaches the advanced stage (pl. 1, *F*), it appears to enter a state of semiarrested development. During the remainder of the current growing season there is only a slight increase in diameter of the carpels, and there is no development of the carpel lobes to cover the locule cavities. Development of the carpel lobes to form stylar and stigmatic tissues is definitely deferred until the following spring.

SPRING GROWTH OF FRUIT BUD

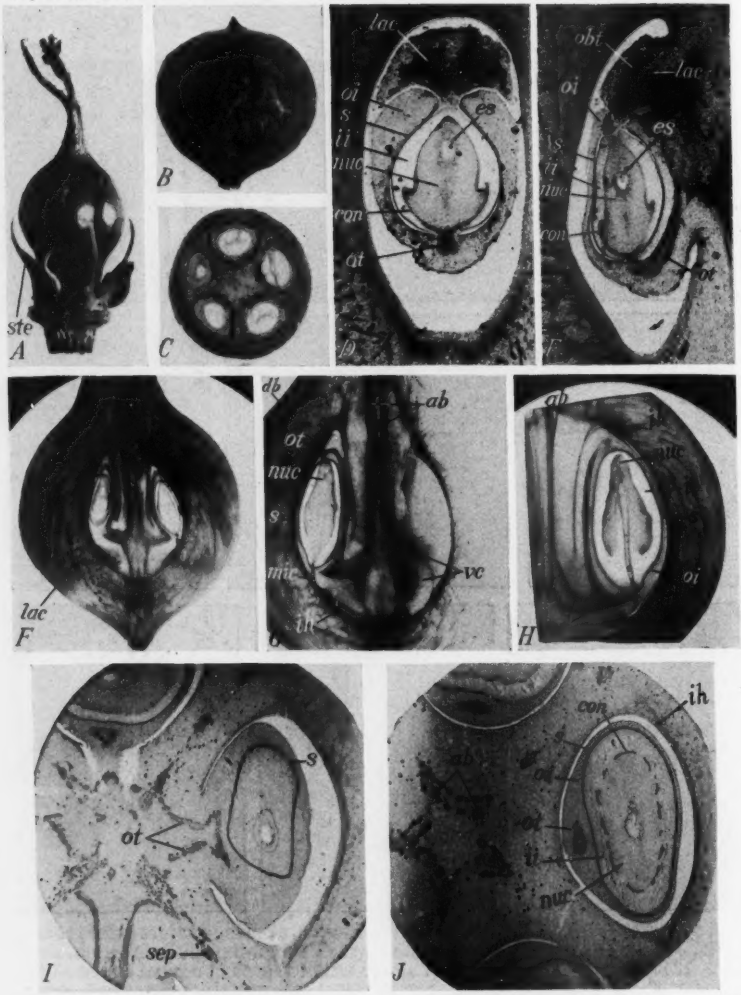
After the winter dormant period, the pistillate flowers resume rapid activity (pl. 1, *G–J*). In 1939 growth began in early February, and between February 4 and March 15 the pistil increased in diameter approximately sevenfold. Activity can first be observed in the lengthening of the carpellary lobes (pl. 1, *G* and *H*) and of tissues of the central axis or placenta and septal walls. These soon meet and close over the locular cavities (pl. 1, *I*). Stylar and stigmatic tissues (pls. 1, *I* and *J*, and 2, *A*) are formed from the extension of the pericarp.

ENLARGEMENT OF FRUIT

Vigor of the tree, the number of carpels per ovary, and the position of the pistillate flower in the inflorescence all have a bearing on the size of the developing pistillate flowers. In addition, the time of initiation has an important influence on the size that the pistillate flower attains



A, Top view of early stage of flower-bud initiation, showing distinct sepal primordia, June 12, 1940. *B*, Determinate stage of flower-bud differentiation, June 25, 1940; petal and staminode primordia initiated around central meristematic dome of pistillate flower. *C*, Development of central meristematic dome, July 8, 1940; carpel primordia appearing on periphery. *D*, Stamen primordia formed spirally over meristematic surface, July 12, 1940. *E*, Pistillate flower in cup stage, November 4, 1939; definite locule formation in the carpels, but no development of placenta evident. *F*, Top view of terminal bud showing young advanced stage of pistillate flower surrounded by young male flowers, July 25, 1939. ×6.5. *G*, Pistillate flower showing development of carpels and increase in diameter, February 19, 1939. *H*, Further growth of carpels; locules outlined by growth of septal regions of fruit, February 20, 1939. *A* to *E*, *G* and *H*, Photographed with microtessar lens (48 mm.). ×26. *I*, Development of stylar and stigmatic tissues, March 7, 1939; stigma lobe of each carpel bifurcated. ×7. *J*, Pistillate flower collected at time of full bloom, March 17, 1939. ×1. *car*, carpel; *pe*, petal; *se*, sepal; *sta*, stamen; *ste*, staminode.



A, Young fruit collected about 1 week after full bloom, March 23, 1939. ×1.5. *B*, Mature, dry fruit showing slight basal septicidal dehiscence. ×0.5. *C*, Cross section of fresh fruit showing variation in the amount of filling of the seeds, August 5, 1940. ×0.5. *D*, Tangential longitudinal section through an ovule of a fruit, March 17, 1939 (from a prepared slide). ×16.5. *E*, Median radial longitudinal section through an ovule of the same fruit as in *D*. ×16.5. *F–H*, Glycerin-cleared sections of fresh fruits showing vascular connections to ovules: *F*, Four-carpelled fruit, April 21, 1939. ×1½; *G*, five-carpelled fruit, April 27, 1939; ×2; *H*, one carpel of fruit, May 17, 1939; ×1½. *I* and *J*, Fixed and stained sections of fruit showing ovular traces branching from the vascular complex, April 21, 1939; ×9.5. *ab*, Axis bundle; *con*, conducting tissue (seed); *db*, dorsal bundle; *es*, embryo sac; *ih*, inner hull; *ii*, inner integument; *lac*, latex system; *mic*, micropyle; *nuc*, nucellus; *obt*, obturator; *oi*, outer integument; *ot*, ovular trace; *s*, shell of seed; *sep*, septal bundle; *ste*, staminode; *vc*, vascular complex.

before winter. Pistillate flowers initiated late in the summer reach the cup stage, but as a rule do not attain the diameter of buds initiated earlier in the season. Here again an accurate comparison is difficult because of differences between individual flowers on the same tree and between individual seedling trees. Just before anthesis the ovary attains an average diameter of 5.4 mm. or about seven times the diameter at midwinter.

The diameter of the ovary at the time of full bloom (pl. 1, *J*) ranges from 6 to 15 mm. Table 2 contains growth measurements made at approximately monthly intervals on killed and on growing tung fruits. The 1940 season was marked by a late spring and a consequent late blooming period. Subsequent growth of the fruits was also later in starting in 1940 than in 1939, but it is also true that the rate of growth was faster in the late-blooming year than in the early-blooming year. In both seasons growth in size was complete by about July 15. Growth of endosperm and embryo does not start until after the fruit has practically attained its maximum diameter.

GROWTH PERIODS OF FRUIT

From the time of fruit set to maturity, the growth of the tung fruit can be divided into two major periods: (1) A period of growth in size of the structural elements of hulls and seed coats and (2) structural and physiological filling of the seed by growth of the endosperm and growth of the embryo. This period is initiated at the time of or shortly before maximum size of the fruit is attained.

TABLE 2.—*Diameters of tung fruits on different dates*

Date	1939 [1]	1940 [2]	Date	1939 [1]	1940 [2]
	Millimeters	*Millimeters*		*Millimeters*	*Millimeters*
Mar. 15–25	6–15		June 10–20	50–55	36–51
Apr. 10–20	20–30		July 10–20	56–60	52–59
May 10–20	35–45	16–29			

[1] From random samples of killed fruits.
[2] From weekly measurements of a total of 250 fruits on 16 trees.

STRUCTURAL DEVELOPMENT OF SEED

The diagrams of figure 2 represent structural development of the seed at weekly intervals from June 10 to August 12, 1940. Each figure at each date represents the average of the seeds in 30 fruits collected at random. There is wide variation in seeds even within the same fruit (pl. 2, *C*); likewise between fruits on the same or different trees (pl. 3, *A*). The cross-section and longitudinal-section diagrams for June 10 show the young seed still in the process of growth and development (fig. 2, *A*, *a*, and *B*, *a*). The shell was thickening but did not begin to harden until about July 1. It is formed from the outer epidermis of the inner integument and encloses the remainder of this tissue. The shell is completely surrounded by outer integument tissue. The embryo sac is embedded in nucellar tissue at the micropylar end of the ovule, and the nucellus is surrounded by the inner integument. The embryo sac is lined by a thin peripheral layer of endosperm.

GROWTH OF EMBRYO

In early June the embryo and the endosperm are still microscopic. Increase in size of both begins early in July, at the time of or just before cessation of diameter growth in fruits. The longitudinal-section diagram for July 1 (fig. 2, *B*, *d*) shows a small globular embryo at the micropylar end of the embryo sac. It is surrounded by the

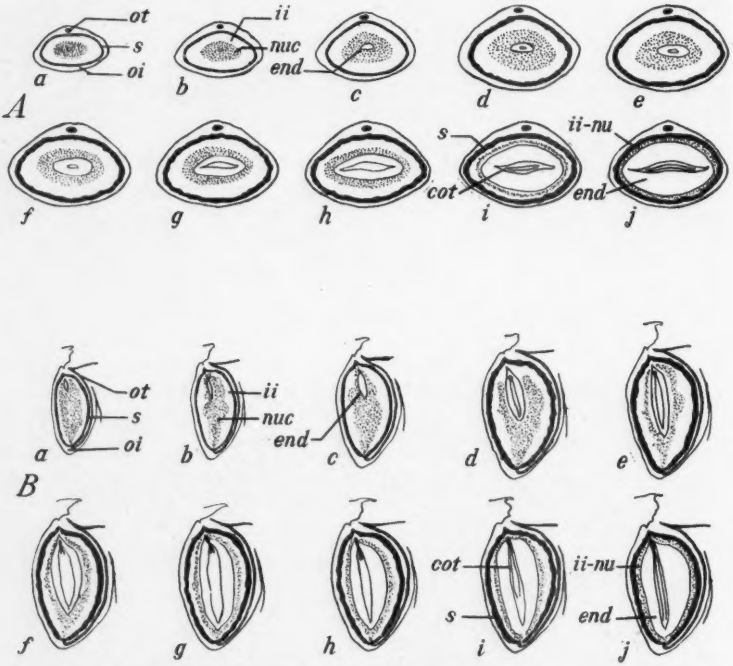
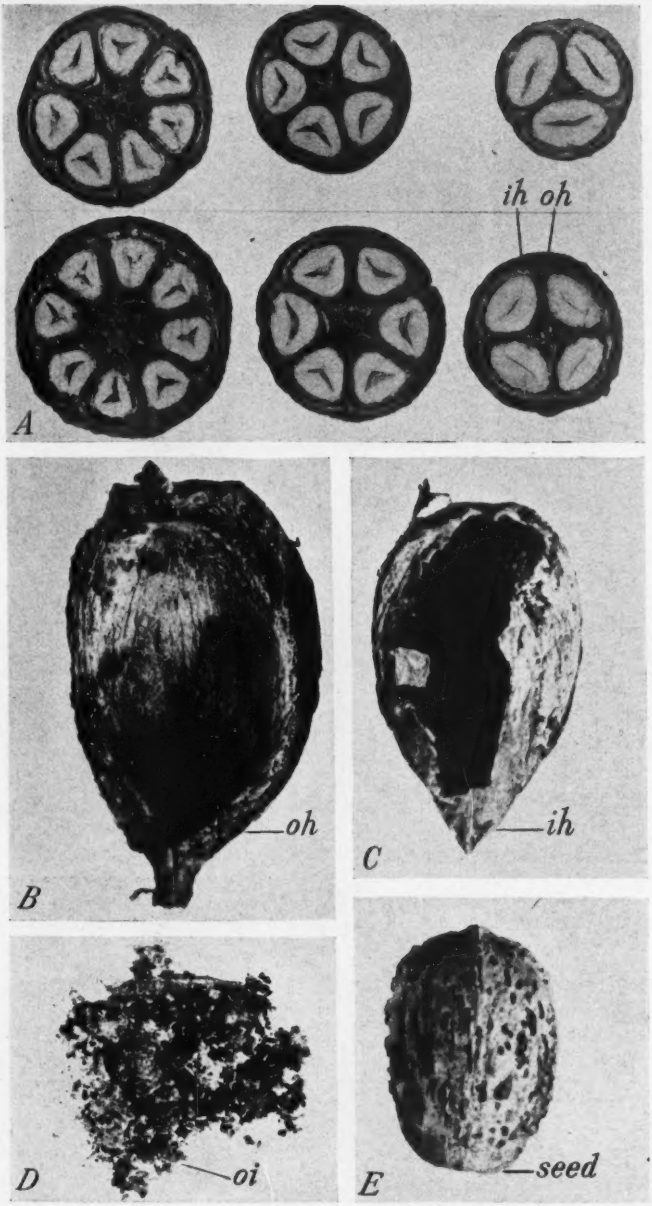
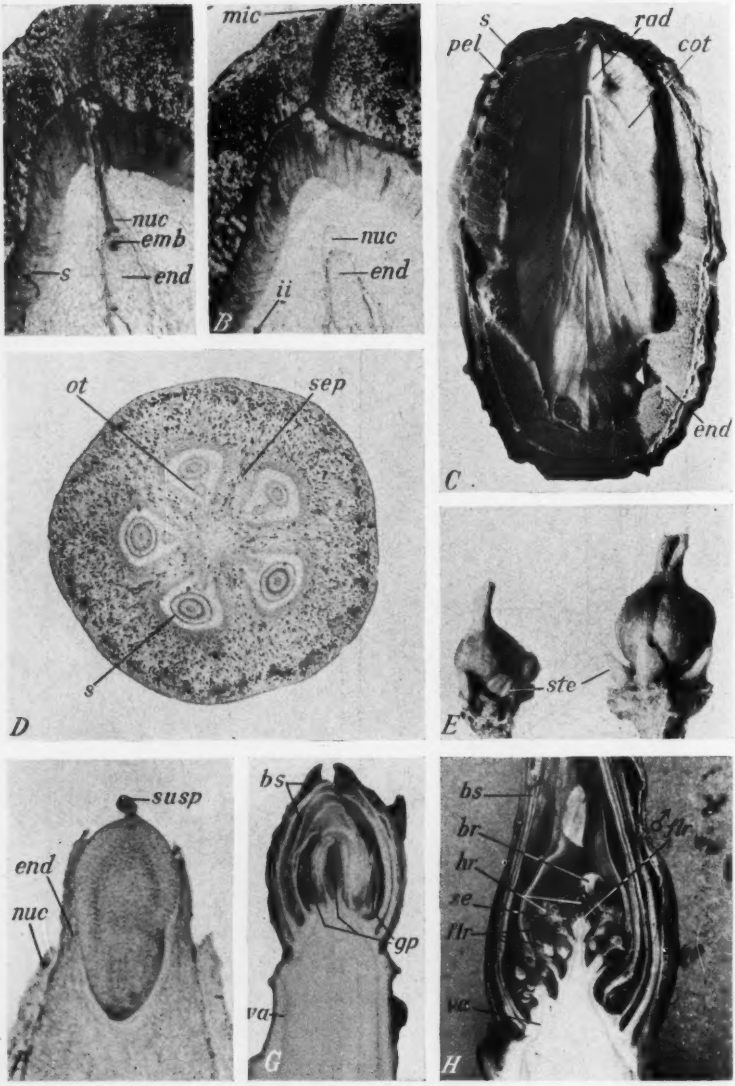


FIGURE 2.—Weekly stages of endosperm and embryo growth between June 10 and August 12, 1940. ×0.5. *A*, Median cross sections of the seed: *a*, June 10; *b*, June 17; *c*, June 24; *d*, July 1; *e*, July 8; *f*, July 15; *g*, July 22; *h*, July 29; *i*, August 5; *j*, August 12. *B*, Median radial longitudinal sections: *a*, June 10; *b*, June 17; *c*, June 24; *d*, July 1; *e*, July 8; *f*, July 15; *g*, July 22; *h*, July 29; *i*, August 5; *j*, August 12. Diagrams showing the cross and longitudinal sections for July 1 (*A*, *d*, and *B*, *d*) indicate the initiation of rapid growth, which occurs after the maximum diameter of the seed is reached. The longitudinal section also shows the first macroscopic evidence of embryo growth near the micropylar end of the seed as a small globular mass of tissue, entirely surrounded by endosperm. The succeeding diagrams indicate the growth of the endosperm and embryo at the expense of inner integuments and nucellar tissue. *cot*, Cotyledon; *end*, endosperm; *ii*, inner integument; *ii-nu*, remains of inner integument; *nuc*, nucellus; *oi*, outer integument; *ot*, ovular trace; *s*, shell of seed.

peripheral endosperm of the embryo sac. The embryo is attached by its suspensor to the micropylar end of the embryo sac (pl. 4, *A* and *F*). Rapid growth of the cotyledons was observed between July 1 and August 12. As endosperm tissue develops, the embryo sac lengthens toward the chalazal end of the seed, and by August 12 the cotyledons extend to almost the full length of the ovule. The



A, Median cross sections of dry mature fruits, showing variation in number of seeds per fruit. ×0.75. *B-E*, Various parts of one carpel (section) of mature tung fruits: *B*, Outer hull; *C*, inner hull; *D*, "dust" removed from seed coat, dry outer integument tissue; *E*, seed. ×1.5. *ih*, Inner hull; *oh*, outer hull; *oi*, outer integument.



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two thin cotyledons lie in a longidtuinal plane parallel to the wide axis of the seed (pl. 4, *C*). As the fruit hangs on the tree, the apex is directed downward. The radicle of the embryo is directed toward the apex of the fruit.

Beginning about July 1, 1940, 30 fruits were collected at random from each of 2 orchards at weekly intervals. Each week embryo measurements were obtained by dissecting out 1 embryo per fruit from 10 different fruits from each orchard. These data, given in table 3, show that the embryo was large enough to be visible under the dissecting microscope about July 1 and that its most rapid growth occurred about 1 month later.

TABLE 3.—*Length of tung embryos at various dates in 1940*

[Each figure is an average of 10 embryos from different fruits]

Date	Plot 1	Plot 2	Date	Plot 1	Plot 2
	Millimeters	*Millimeters*		*Millimeters*	*Millimeters*
July 1	0.00	0.24	Aug. 2–5	4.32	11.91
July 5–8	.71	.73	Aug. 9		14.78
July 12–16	1.05	1.47	Aug. 12		15.10
July 19–23	2.16	2.77	Aug. 16–19	18.79	20.14
July 27–29	3.37	4.74			

GROWTH OF ENDOSPERM AND STRUCTURAL FILLING OF SEEDS

The endosperm (pl. 4, *A* and *B*, and fig. 2) begins growth soon after fertilization. There is a small but constant increase of endosperm tissue in the seeds while the fruit is rapidly increasing in size. Slightly before the attainment of maximum fruit size, the endosperm begins rapid centrifugal growth (fig. 2). By the middle of August this tissue, which contains the commercial tung oil, has filled the seed except in the region immediately adjacent to the inside of the shell. In this connection it should be pointed out that although the seeds are filled with endosperm tissue 6 to 8 weeks after maximum fruit size is attained, the cells of this tissue still contain very little tung oil.

The growth of endosperm described above has been made at the expense of the nucellus and inner integument tissues. By early June the nucellus fills a large proportion of the space within the seed coat or shell. The nucellus has the appearance of a translucent, hyaline

A, Micropylar end of seed showing embryo still microscopic in size, and endosperm; shell developed from outer layer of inner integument; outer integument surrounds shell; June 21, 1940. ×14. *B*, Same as *A*; section succeeding *A* showing dark-staining cells in micropylar canal between the outer integuments. ×14. *C*, Longitudinal section showing mature seed and seed coat with reticulate paperlike pellicle; endosperm filling major part of seed in which is enclosed the young embryo; upper cotyledon has been removed. ×2.5. *D*, Cross section just below vascular complex of young fruit, March 15, 1939. ×14. *E*, Buds, March 5, 1939; young pistils prior to anthesis showing development of staminodes and apparently normal anthers. ×3. *F*, Young embryo, approximately 1 mm. long, from seed, July 18, 1940. ×33. *G*, Longitudinal median section through 1-year-old terminal bud, showing vegetative terminal meristem, February 15, 1940. ×1.5. *H*, Terminal bud from 8-year-old tree, February 5, 1940. ×3. *br*, Bract; *bs*, bud scale; *cot*, cotyledon; *emb*, embryo; *end*, endosperm; *flr*, flower; *gp*, growing point; *hr*, hairs; *ii*, inner integument; *mic*, micropyle; *nuc*, nucellus; *ot*, ovular trace; *pel*, pellicle; *rad*, radicle; *s*, shell of seed; *se*, sepal; *sep*, septal bundles; *ste*, staminode; *susp*, suspensor; *va*, vascular system.

gel in contrast with the integument tissue, which remains opaque. As endosperm filling progresses, constant encroachments are made on nucellus and integument tissues until by mid-August only a thin layer remains between the endosperm and the shell. Remnants of nucellus and integument form the pellicle in the mature seed.

STRUCTURE OF THE MATURE FRUIT

At the time of fruit drop, usually late October or November, the hull is soft and somewhat pulpy and the fruit bears a striking resemblance in shape and color to a small apple. The majority of the fruits are oblate-spheroid in shape, but a few are ovoid, obovoid, and top-shaped. The color ranges from green to a dark maroon. Some fruits exhibit a red blush of varying degrees of intensity over a green ground color. After dropping from the trees, the fruits in the commercial orchards are allowed to dry on the ground for a time and are then picked up by hand and placed in storage sheds or bins to dry thoroughly before milling.

A thoroughly dry fruit has a dark-brown and subwoody hull, which exhibits a slight tendency toward basal and more rarely toward apical septicidal dehiscence (pl. 2, *B*). Each carpel is composed of a thick, fibrous, subwoody, boat-shaped outer hull (pl. 3, *B*) and an inner hull which lies in the concavity of the outer hull and envelops the seed with the exception of the funicular region (pl. 3, *C*). The inner and outer hulls separate readily. The dorsal periphery of the inner hull is thicker and tougher than the brittle, parchmentlike radial edges, which readily tear and permit the enclosed seed to be freed. The rough exterior of the seed coat is covered with a fine dust, the remains of the outer integuments.

The mature seed (pl. 4, *C*) consists of a rough, bony seed coat about 1 mm. thick; the pellicle, which is a thin reticulate, paperlike layer immediately inside the shell; the endosperm, which composes the major part of the kernel; and the embryo, which is enclosed with the endosperm. The embryo consists of a comparatively large radicle and two thin flat cotyledons.

VASCULAR ANATOMY OF FRUIT

Vascular studies were made on the developing pistillate flowers from the time of their initiation to the time when the fruits attained their maximum size. In early stages of flower-bud formation, separate and distinct traces diverge from the main axis to sepals, petals, staminodes, and carpels (fig. 3).

Green fruits were collected on August 15, 1940, after maximum size had been attained and seed filling was almost complete structurally. Studies of sections of these green fruits, together with previous studies made on glycerin-cleared and pine-oil-cleared fruits, gave data from which to construct a diagram of the vascular system as of August 15, 1940 (fig. 4). The fruit is shown in cross section along seven horizontal planes (*C* to *I* inclusive), in median longitudinal section through a seed (*A*), and in longitudinal section through a septum (*B*). It will be noted that in the peduncle there is a ring of vascular tissue at *C*, which breaks into strands above *D*. The number of strands depends upon the number of carpels in the fruit. The first strand to

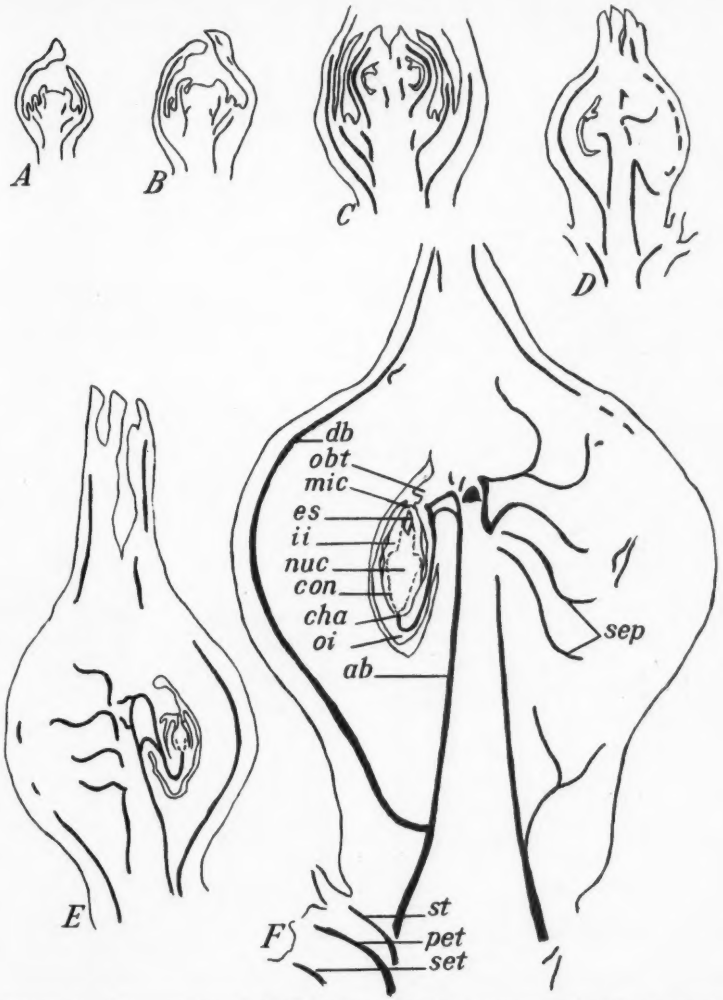

FIGURE 3.—Microprojector drawings of longitudinal sections made from prepared slides showing the development of the pistillate flower from the dormant bud to fruit setting. ×7. *A*, Dormant pistillate flower, January 5, 1939. *B*, Initiation of spring growth, February 5, 1939; all parts have grown. *C*, Carpellary tissue developed and covering locular cavities, February 22, 1939; ovule development beginning. *D*, Further development of carpels to form stylar tissue, March 5, 1939. *E*, Pistil just prior to anthesis, March 15, 1939. *F*, Young fruit, April 1, 1939, approximately 1 week after full bloom. *ab*, Axis bundle (septal and ventral undiverged); *cha*, chalaza; *con*, conducting tissue (seed); *db*, dorsal bundle; *es*, embryo sac; *ii*, inner integument; *mic*, micropyle; *nuc*, nucellus; *obt*, obturator; *oi*, outer integument; *pet*, petal trace; *sep*, septal bundle; *set*, sepal trace; *st*, staminode trace.

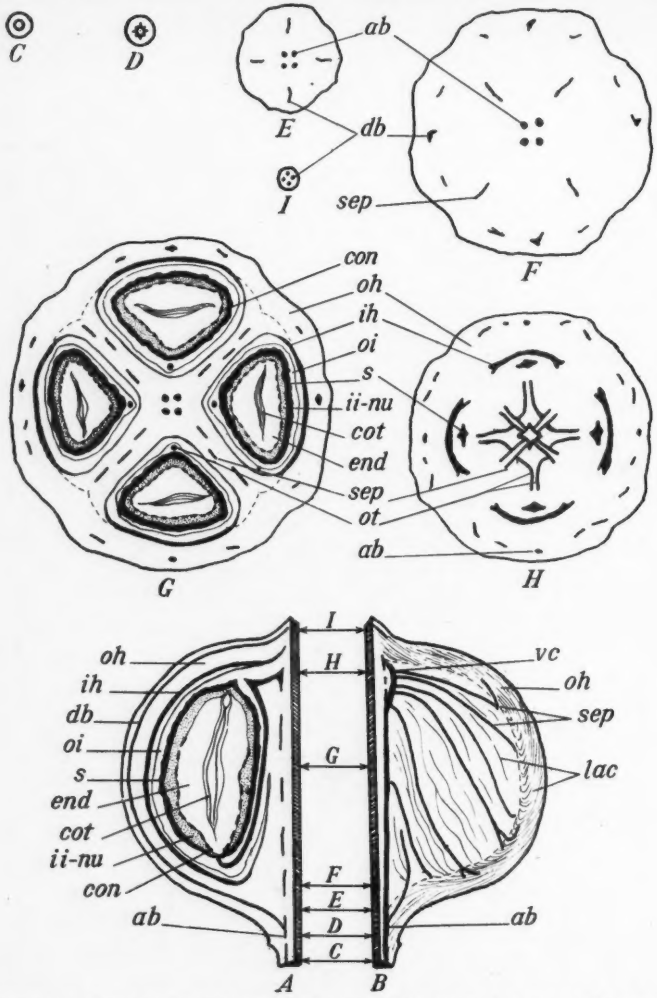

FIGURE 4.—Vascular study of tung fruit, August 15, 1940, ×0.75: *A*, Median longitudinal section through one carpel; *B*, longitudinal section through septal region; *C–I*, cross sections at horizontal planes indicated between *A* and *B*. At plane *C*, the peduncle contains a ring of tissue; at plane *D*, dorsal carpellary bundle diverges from vascular ring; at plane *E*, four strands of axis-septal bundles in placental region; at plane *F*, branching of dorsal carpellary bundles; at plane *G*, median cross section of fruit shows four axis-septal bundles in placental region, ovular traces in ventral side of outer integument of each seed, and parts of several septal bundles in septal regions between seeds; dorsal branches have diverged; at plane *H*, axis-septal bundles of placenta terminate in vascular complex; divergence of ovular traces; at plane *I*, four dorsal bundles at base of style. *ab*, Axis bundle (septal and ventral undiverged); *con*, conducting tissue (seed); *cot*, cotyledon; *db*, dorsal bundle; *end*, endosperm; *ih*, inner hull; *ii-nu*, remains of inner integument; *lac*, latex system; *oh*, outer hull; *oi*, outer integument; *ot*, ovular trace; *s*, shell of seed; *sep*, septal bundles; *vc*, vascular complex.

diverge from the main axis, just below cross section *E*, is the dorsal bundle. The main dorsal bundle branches to form several traces scattered throughout the mesocarp tissue. In cross section *I*, at the base of the style, there are four dorsal bundles, one for each carpel.

The axial strands continue up the central axis of the fruit to a vascular complex near the apex (fig. 4, *B*). The number of main axial bundles in the placenta depends on the number of carpels in the fruit. Thus, there are four in the four-carpelled fruit and five in the five-carpelled fruit. These strands (pl. 2, *G–I*) may be interpreted as undiverged axis-septal bundles. The septal regions and the ovules are supplied from the vascular complex (pl. 2, *G*). A number of the septal bundles are shown in the longitudinal section in figure 4, *B*, and in cross sections *F*, *G*, and *H*. Each ovular trace is formed by two strands that diverge from separate septal bundles just after the latter leave the vascular complex (pl. 2, *I*, and fig. 4, *H*). Each ovule receives vascular supply from the two adjacent septal regions (fig. 4, *H*). The strands from the two septal regions fuse to form an ovular trace (pl. 2, *G–J*, and fig. 4, *A*, *G*, and *H*). The trace follows the outer integument along the ventral side of the seed and enters it at the chalaza (pl. 2, *D* and *E*, and fig. 3, *F*), connecting to tissue within the seed that stains darkly in the early stages of development and later develops spiral elements characteristic of conducting tissue (pl. 2, *J*). Thus the nucellus, developing endosperm, and embryo are in direct contact with the main vascular system of the fruit and tree until filling of the seed by endosperm is complete.

At the micropylar end of the seed the ovular trace may be seen to follow the outer integument around the seed toward the micropyle. However, the trace stops short of reaching the micropyle (pl. 2, *G*, and fig. 2, *B*, *a*).

In the early development of the ovule (fig. 3, *E*) the nucellus extends beyond the inner and outer integuments in the form of a long bottle-necked structure, which seems to be connected with the obturator. Both the obturator and the projected nucellus evidently serve for osmotic conduction during the early stages of ovule development. Both structures gradually lose their identity as the shell forms from the outer layer of the inner integument.

DISCUSSION

PERIOD OF FRUIT-BUD DIFFERENTIATION

The extensive period of time through which fruit-bud initiation may take place as reported by Abbott (*1*) has been corroborated in this work. In 1926 and 1927 Abbott found that fruit-bud differentiation in the tung tree occurred at any time between May 10 and October 1, with the majority of the buds differentiating during the latter half of June. In the present study the earliest evidence of fruit-bud formation was found during the last week of May 1939 and the first week of June 1940. Lateral pistillate flowers were found in the determinate stage as late as the latter part of September 1939 and 1940.

The variation in time of formation of fruit buds is probably caused by a number of factors. Abbott (*1*) asserted that a shortage in the amount of available soil moisture will materially hasten time of differentiation. Many growers of deciduous fruits believe that dry weather is favorable for the formation of fruit buds. There is little definite

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evidence on the matter. Degman and others (*7*) found no correlation between moisture supply and fruit-bud formation in the Oldenburg apple, while in Rome Beauty the trees that became dry formed a greater number of fruit buds. Rasmussen (*17*) reported that blossom-bud formation in Baldwin and McIntosh apples occurred earlier and was more abundant in the rather dry season of 1929 than during the preceding season in which there was heavy rainfall.

In contrast with the long period of differentiation in tung, Rasmussen (*17*) found that in New Hampshire the majority of the buds on fruit spurs of the apple seem to be initiated during a period of only 10 to 20 days. The period of differentiation for buds on terminal shoots of apples is doubtless longer. In the apple there are relatively few of these blossoming terminal shoots, whereas tung bears all its fruits terminally on shoots.

While the earliest date of flower-bud formation has been determined in many fruits, the period during which the majority of the buds are initiated has not received adequate attention. Hence, it is not possible to make further comparisons between tung and other fruits. The extended period of flower-bud formation in tung is under further study to determine what environmental factors may have a bearing on the date of initiation and the rate of development of the flower.

OVERWINTERING PISTILLATE BUDS

Woodroof (*25*, *26*) reported that the pistillate flower of pecan is initiated in February, while the staminate catkins are definitely formed the summer before. Therefore, the pistillate bud of the pecan is not recognizable as such during the dormant season. During the dormant period the pistillate flowers of tung can easily be identified (pl. 1, *E* and *F*) and can be distinguished from the staminate flowers (pl. 1, *D* and *F*), which may occur in varying degrees of development within the same terminal bud.

GROWTH PERIODS OF TUNG AND OTHER FRUITS

The total growth in diameter of the tung fruit was plotted against time by Kilby and Parker (*10*). A typical sigmoid curve was obtained. The hull and seed coat reach maximum size at the end of a single cycle of growth activity. The embryo remains microscopic until about the time that the fruit attains maximum size. Immediately after the first cycle of growth in size, a second period of growth is begun during which the filling of the seeds with endosperm and the growth in size of the embryo occur. Hence, the growth periods of tung are similar to those of the almond as reported by Brooks (*4*), and to those of the pecan as described by Thor and Smith (*20*) and Crane and Hardy (*5*). The growth of the tung fruit differs from the growth of fleshy drupe fruits as reported in apricot by Lilleland (*13*), in cherry by Tukey (*22*) and Tukey and Young (*24*), and in peach by Tukey (*21*), Tukey and Lee (*23*), Harrold (*9*), and Lilleland (*14*). There is no appreciable final swell of the mesocarp in tung such as is found in the fleshy drupes mentioned.

Growth of the almond, a dry drupe, as reported by Brooks (*4*), is essentially similar to that of tung. It reaches its maximum size at the end of a single cycle of growth activity. During this period the

seed coats reach their full size, but the embryo remains microscopic. The first growth cycle is followed by a second in which embryo growth and filling of the nut take place. Because the mesocarp remains a dry tissue, there is no final swelling of the fruit as in fleshy drupes.

As a whole the development of the tung fruit and filling of the seeds are analogous to the condition in pecan as reported by Thor and Smith (*20*) and Crane and Hardy (*5*). The first period of growth in pecan is from blossoming to late August or early September and is characterized by formation of structural elements of shuck and shell, while during a later second period filling of the kernel is of major importance. The first period of growth in the tung fruit is from time of blossoming to the time of maximum fruit enlargement—the structural development phase. The second period covers the filling phase and includes the major growth of endosperm and embryo and subsequent physiological changes in the endosperm. It is possible that the second period may be subdivided by further chemical studies of the kernel subsequent to the completion of the structural phase.

VASCULAR SYSTEM OF TUNG FRUIT

From the time of the formation of the embryo sac, the ovule is connected, by means of differentiated conducting tissues, with two sources of elaborated materials, namely, the leaves and the chlorophyll-bearing tissue of the outer hull. The undiverged axis-septal bundles, leading directly from the vascular system of the stem, pass through the center of the tung fruit from the base toward the apex and terminate in a vascular complex in the placental region. A considerable number of septal bundles lead from this vascular complex and extend through the parenchymatous tissue between the carpels. Each axis-septal bundle of the placenta supplies two adjacent ovules in part. Strands that are seen to diverge from septal bundles just after they leave the vascular complex converge in the funiculus of each ovule to form the ovular trace, which enters and follows the ventral side of the outer integument to the chalazal end of the ovule or seed and connects with conducting tissue within the seed coat.

Substances elaborated or stored in the outer hull can be transported to the axis bundles through the dorsal bundles and their branches or may in part move directly from the hull through the septal bundles to the ovular traces.

STRUCTURE OF MATURE FRUIT

The fruit of the tung tree is morphologically distinct in that it is not readily allocated to any specific group of the conventional classifications of botanical fruits.

Detailed comparison of the tung fruit with other members of the Euphorbiaceae is difficult because of the wide divergence of form and structure within the family. The plants of the family that do bear fruits similar to that of tung are for the most part tropical, are not grown widely in the United States, and are not described in the literature on fruit morphology. This is especially true of the remaining members of the genus *Aleurites:* namely, *A. montana* (Lour.) Wilson, *A. trisperma* Blanco, *A. moluccana* (L.) Willd., and *A. cordata* (Thunb.) Muell. Of the better known plants of the Euphorbiaceae, the castor-bean (*Ricinus*), as described by Bailey (*3*) and Small (*19*), warrants

mention in that its fruit probably most closely resembles the tung fruit in general structure.

The husked fruits of the Juglandaceae, specifically the pecan and the walnut, have certain extrinsic similarities to the tung fruit. However, their morphological structure is vastly different. According to the studies of Langdon (*11*, *12*), Nast (*16*), Shuhart (*18*), the Woodroofs (*25*, *26*, *27*, *28*, *29*), and Adriance (*2*), the husks of the fruits of the Juglandaceae are composed of receptacle and perianth tissues, which are floral appendages, while the hull of the tung fruit is a development from ovary-wall tissues. The shells of the pecan and the walnut are lignified parts of the ovary walls, while that of the tung is lignified outer epidermis of the inner integument. The so-called packing tissue of the pecan and walnut, which is found inside the shell, is homologous to the parchmentlike inner hull that surrounds the seeds of the tung fruit. The kernel of the tung seed is mainly endosperm, but those of the pecan and the walnut are embryos.

SUMMARY

Tung trees are commonly monoecious and predominantly synoecious but trees producing preponderantly staminate or pistillate inflorescences are not uncommon. The tung tree also shows a tendency to produce perfect flowers. Blossoms are initiated in terminal buds on shoots of the current season. Flowers are produced in cymose inflorescences from a terminal pulvinate growing point. New shoot growth usually arises from buds immediately below the flower stalks.

The inflorescence of the tung tree is highly variable in number and position of the pistillate flowers. Any one or all of several types of inflorescence may be found on any one tree, but there is a tendency for one type to predominate. Thus, certain trees can be termed "single," "bunch cluster," "grape cluster," "staminate," or "pistillate," depending upon the nature of the inflorescence.

Pistillate flowers are initiated from late May to late October. The development of the inflorescence is basipetal, but that of the individual flowers is acropetal.

The dormant winter or resting bud contains pistillate flowers in which the carpels have developed to form a cup-shaped structure. Development of the carpel walls to form stylar and stigmatic tissues does not begin until the initiation of spring growth, usually about the first part of February. By the time of anthesis, increase in diameter is about sevenfold.

From the time of fruit set to maturity, the growth of the tung fruit can be divided into two major periods: (1) A period of growth in size of the structural elements of hulls and seed coats and (2) structural and physiological filling of the seed by growth of the endosperm and growth of the embryo. This period is initiated at the time of or shortly before maximum size of the fruit is attained.

Each carpel of the normally five-carpelled fruit is composed of a thick, fibrous, subwoody outer hull, in the concavity of which is found a parchmentlike inner hull, which almost completely encloses the seed. The inner and outer hulls separate readily. The mature seed is covered with a layer of dust, the remains of the outer integument. The seed consists of a rough, bony seed coat (a development of the outer epidermis of the inner integument); a thin, reticulate, paperlike

pellicle immediately inside the shell (the remains of inner integument and nucellus tissue); the endosperm, composing the major portion of the kernel (the source of the commercial tung oil); and the embryo, consisting of a comparatively large radicle and two thin flat cotyledons.

Vascular studies of the developing pistillate flower and growing fruit show that separate bundles diverge from the main axis to sepals, petals, staminodes, and carpels. Studies of the vascular system of the mature fruit show that bundles are arranged in a cylinder in the fruit stem or peduncle. The cylinder breaks into strands in the "neck" of the stem end of the fruit. Dorsal carpellary branches leave the main axis just above the "neck" and traverse the length of the carpels through the mesocarp tissue into the stylar tissue. The axis bundles continue up the center of the fruit along the septal radii of the axis or placental region as undiverged axis-septal bundles terminating in a vascular complex in the funicular region. Each axis-septal bundle contributes branches to each of two ovules. Strands diverge from each side of each axis-septal bundle and converge in the funicular region to form the ovular trace of each carpel. The septal regions between the seeds are supplied with a considerable number of strands, which connect the outer hull with the vascular complex of the placenta.

The fruit of the tung tree is morphologically distinct and is not readily allocated to any specific conventional classification such as dry drupe or capsule.

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MEASURES OF GROWTH RATE FOR USE IN SWINE SELECTION[1]

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INTRODUCTION

Rapid growth of swine is of value to the pork producer for several reasons. It is generally agreed that other things being equal faster-growing pigs require less feed per unit gain in weight. Since the pigs that grow faster reach market weight sooner the overhead cost of labor is smaller and the risk of loss from disease, parasites, and accidents is reduced. In northern climates where cold weather often occurs late in the spring and early in the fall, rapid growth makes it possible to raise a crop of pigs during the short season when there is relatively little chance of their being subjected to severe weather. It follows that growth rate is one of the characters to be considered in any improvement program.

The studies to be reported were made with data collected on swine raised in the United States Regional Swine Breeding Laboratory project at the University of Minnesota and its substations. The swine in question were largely of the Poland China breed. However, from a quarter to a third of the data used in studying postweaning growth and the heritability of growth rates were obtained on a strain designated as Minn. No. 1. This strain has been developed by inter se mating of pigs from a cross of the Tamworth and Danish Landrace breeds, and on an average the 1940 crop was about three generations removed from their F_1 ancestors. All the pigs have been moderately inbred since the lines were established in 1937. In 1940 the coefficient of inbreeding (calculated according to Wright (*21*)[2]) averaged about 25 percent for the Poland China and 20 percent for the Minn. No. 1 hogs.

GROWTH OF PIGS UP TO 56 DAYS OF AGE

Since the value of phenotypic selection in the choice of breeding stock depends on the extent to which observed differences result from differences in genotype, it is desirable to control the environment of animals from which selections are to be made in a manner that will both minimize nongenetic variation and promote complete expression of genotypic differences. Thus selection for growth rate will be most successful when all animals receive as much feed of an optimum quality for growth as they can utilize. If the amount of feed provided is less than the animals can use or if the quality is not optimum, full expression of genotypic differences will not be obtained. If, in addi-

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[2] Italic numbers in parentheses refer to Literature Cited, p. 388.

tion, the amount or quality of feed available varies from one animal to another, a source of nongenetic variation in growth is introduced.

Donald (*6*) states, regarding sows, that different gland sections of the udder may secrete very different quantities of milk. The statement is based on his own data reported in another paper (*5*). He also found (*6*), as did Bonsma and Oosthuizen (*1*) and Carlyle (*4*), that, in general, each pig nurses the same teat throughout the suckling period. Considering these things together, he concludes that differences in nutritional opportunity are the source of many of the differences in the weight of pigs at both 3 and 8 weeks, and consequently that weight differences at those ages are a poor indication of differences in genotype for growth. However, since Donald determined milk production by measuring (indirectly through difference in weight of pigs before and after nursing) the amount of milk taken by the pigs in suckling, the possibility remains that the differences observed arose from variation in the appetites of the pigs and not from variation in productive ability of the separate sections of the udder. In effect, this would mean that each pig received as much milk as it would take and that differences in the amount the pigs could use or cared for made it appear that the different glands produced widely different amounts of milk. That each pig in a litter does receive all the milk that it can take hardly seems likely in view of the fact, known to anyone who has carefully observed the nursing process, that pigs over 2 or 3 weeks of age usually appear to want more milk than they are able to get. Nevertheless, more definite information showing that at least some of the pigs in a litter actually receive less milk than they can utilize would help to clarify the issue.

The usual conception of growth is that up to a certain limiting size, as animals become larger, their growth as measured by weight is more rapid if environment is optimum. Brody (*2*) stated this more precisely when he said that the growth of animals tends to follow a sigmoid curve with its point of inflection at or near the time of puberty. In the case of suckling pigs this means that the growth rate should increase from day to day and week to week and, since this period is so far in advance of puberty, the increases in growth rate with unit time should be progressively larger. Otherwise there is reason to believe that environment is not optimum.

Data on the growth of suckling pigs have been collected at the University of Minnesota. These include weights at birth and every 7 days thereafter up to and including the fifty-sixth day, weaning age. Pigs raised at the Central station in 1939 and at the Central and Northwest stations in 1940 were included in the total number, 203, for which weights were recorded.

The weekly weight increments of the suckling pigs are presented in table 1. Instead of the theoretical increase throughout the 8 weeks, these increments first increase, then decrease, and then increase again. Is this significant, or are the decreased gains after a preliminary period of increasing gains no smaller than might be expected through chance deviations from a uniformly ascending curve? Sums of squares of deviations from the mean, a straight regression line, and second- and third-order regression lines are presented in table 2. Straight-line regression reduces the sum of squared residuals by a significant amount only in the data from Central station in 1940. The second-

TABLE 1.—*Mean weekly weight increments from the first to the eighth week inclusive by station and year*

Station and year	Pigs	Mean weight increment for week No.—							
		1	2	3	4	5	6	7	8
Central:	*Number*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*
1939	32	2.05	2.51	3.33	3.53	2.90	2.50	3.25	5.75
1940	63	2.59	3.20	3.75	3.15	2.49	4.00	4.67	6.78
Northwest:									
1940	108	2.24	3.01	2.89	2.77	2.35	2.27	3.16	5.46
Total or weighted average	203	2.32	2.99	3.23	3.01	2.48	2.84	3.64	5.92

order regression in no case gives a significantly better fit than does linear regression. In every case the third-order regression gives a significantly better fit than the second-order regression. For these data the second-order regression when plotted represents weekly gains increasing each week, the increase from week to week being progressively larger. The third-order regression equation when plotted shows weekly gains increasing, then decreasing, and at last increasing again as we proceed from the first to the eighth week. Since the third-order regression gives a significantly better fit than the second, it is reasonable to assume that the decrease in weight gained after about the third week cannot be ascribed to chance alone. This constitutes a deviation from the theoretical curve of growth rates discussed earlier and accordingly suggests that environment was not optimum. There is a general agreement in this respect between these and other published data (*9*, *11*, *14*, *16*, *19*).

TABLE 2.—*Sums of squares of deviations of weekly gains from mean and first-, second-, and third-order regression lines, by station and year*

Station and year	Deviation from—			
	Mean	First order regression	Second order regression	Third order regression
Central:				
1939	9.0033	4.8295	4.1455	0.7578
1940	13.6359	5.5880	2.7036	.8322
Northwest:				
1940	7.6725	5.0450	2.8979	.0534
Weighted average	9.0094	4.7502	2.6795	.0645

The most obvious explanation for the failure of growth rate to increase from week to week as expected is that the pigs were limited nutritionally. Smith (*18*) reported the average daily milk flow, in pounds, of 11 sows by weeks as follows: First week, 5.01; second week, 7.46; third week, 8.40; fourth week, 7.80; fifth week, 5.72; sixth week, 5.77; seventh week, 4.65; eighth week, 3.24. These figures indicate an absolute decrease in milk secretion after the third week, whereas even a steady flow would probably have become inadequate for complete realization of ability to grow as the size of the pigs increased. Data reported by Bonsma and Oosthuizen (*1*) and

by others cited by them agree very well with those of Smith. Figure 1 presents graphically the average weekly gains of the pigs weighed in this study, together with Smith's data on milk flow. The parallelism between growth of pigs and milk flow of sows up to the sixth week is striking. Beginning with the sixth week utilization of the supplementary feed offered in creeps apparently began to more than compensate for the inadequacy of the milk supply of the dams.

It should be mentioned in passing that the sows were in all cases full-fed. Management was designed to effect strict sanitation. Precautions were taken against anemia. Vaccination for hog cholera and castration were performed after the decrease in growth rate.

The evidence presented and the literature cited indicate that growth during at least part of the suckling period is limited by nutri-

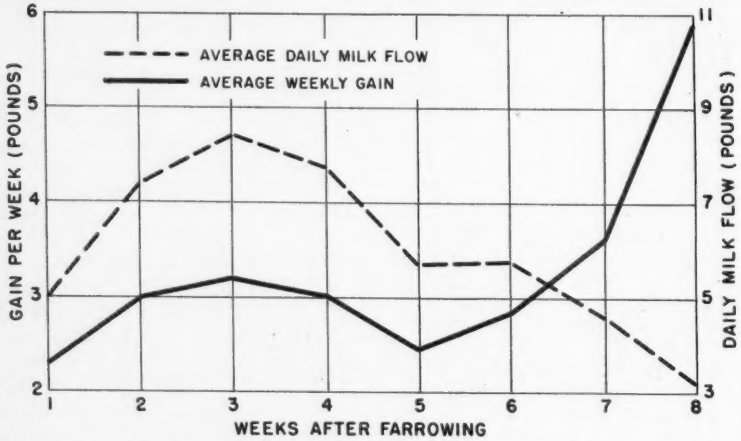

FIGURE 1.—Average daily milk flow of 11 sows, as reported by Smith (*18*), and average weekly gain prior to weaning of 203 pigs.

tion and that the limitations imposed are not equal for pigs in different litters or even for those in the same litter. Under these conditions differences in weaning weight are not likely to correspond very closely to differences in genotype for growth.

Greater persistency of milk secretion by sows would be desirable, but this would not be a complete answer to the problem since the pigs as they grow need not only constant but also increasing amounts of suitable nutrients. If such a method of supplementing the dam's milk could be developed that the growth of suckling pigs would not be hampered by inadequate milk secretion, weaning weight would become more dependent upon genotype and as a result would become more useful in selection for growth rate. The pigs here concerned were creep-fed with either corn and dry-rendered tankage or corn and skim milk. Obviously as supplementary feeds for suckling pigs they leave much to be desired.

If it is true that proper supplementary feeding of pigs prior to weaning would cause weaning weight to be a more reliable measure of the individual's inherited ability to grow, the correlation between weaning

weight and postweaning growth should increase as a result of such feeding. During the years 1938 and 1939, six groups of pigs raised at the Minnesota experiment stations received supplemental feed of some kind during the suckling period while six other groups did not. The intralitter correlation between weaning weight and rate of gain from 50 to 200 pounds was 0.201 for all the pigs that received feed (460 degrees of freedom) and −0.039 for the pigs that received no supplementary feed (439 degrees of freedom). The difference between these correlations has a probability of less than 0.01 of occurrence through chance.[3] This is not as strong evidence of the effect of supplementary feed as it appears to be in view of the fact that in only two cases were both treatments used at the same substation in the same year. However, in support of a theory which appears logical on other grounds it has some significance.

MEASUREMENT OF POSTWEANING GROWTH RATE

In general, the absolute growth rate of normal pigs receiving unlimited amounts of a satisfactory feed increases with body weight at least until the onset of puberty. The effect of this on the measurement of postweaning growth rate is to cause a bias in favor of pigs with a greater initial weight for the period for which rate of gain is measured. Smith and Donald (*17*) pointed this out and presented supporting data. This raises the question whether rate of gain calculated from a standard initial age or from a standard initial weight is the more useful measure of inherited ability to grow during the postweaning period.

Two measures of postweaning growth have been calculated for all pigs raised in the Regional Swine Breeding Laboratory project at Minnesota. They are rate of gain from weaning to 200 pounds (y) and rate of gain from 50 to 200 pounds (y_1). The first equals (200—weaning weight) divided by (age at 200 pounds−56, weaning age); the second equals 150 divided by (age at 200 pounds—age at 50 pounds). Ages at 50 and 200 pounds were determined by interpolation after plotting weight against age on suitable graph paper.

Correlations between weaning weight and both measures of rate of gain were calculated on an intrastation, intrayear basis for all spring pigs raised from 1938 to 1940, inclusive. These are presented in Table 3. The correlation between weaning weight and y is for every

TABLE 3.—*Correlation coefficients describing the relation between weaning weight and two measures of postweaning growth rate*

Breed and source of variance	Degrees of freedom	Rate of gain from—	
		Weaning to 200 pounds	50 to 200 pounds
Poland China:			
Between litters	176	0.344**	0.003
Within litters	1,010	.372**	.114**
Minn. No. 1:			
Between litters	62	.217	−.299*
Within litters	421	.171**	−.092

**Indicates $P < 0.01$ that the observed correlation is different from 0 due to chance alone.
*Indicates $P < 0.05$ that the observed correlation is different from 0 due to chance alone.

[3] Significance of the difference between correlations was here and in all later cases tested by transforming to z's according to Fisher (*8*).

case significantly larger than the corresponding correlation between weaning weight and y_1.

There are at least two paths of relationship between weaning weight and rate of gain (y) which affect the correlation between them: (a) growth before and after weaning is controlled by a common genotype though all genes within that genotype may not have the same relative effect in the two periods, and (b) other things being equal, rate of gain is a function of initial weight for the period in which it is calculated. The latter is a cause and effect relationship, the former is not; both favor positive correlation. A standard initial weight was used in the calculation of y_1 in order to eliminate the effect of (b). If (b) is the only cause and effect relationship between weaning weight and y, y_1 should (1) show less correlation with weaning weight than y (as noted above), and (2) should be less variable than y. If (a) were the only path of relationship between weaning weight and y_1 the correlation between them should lie somewhere between 0 and $\sqrt{h^2w.h^2r}$ (where h^2w is the genetic portion of variance in weaning weight and h^2r is the genetic portion of variance in rate of gain after weaning) depending on how close genes for growth come to giving the same relative stimulus to growth before and after weaning. Bywaters (*3*) estimated h^2w as 0.18. Lush (*12*) estimated the additive genetic portion of variance in rate of gain as 0.24. The total genetic portion would be somewhat larger than this. However, $\sqrt{0.18 \times 0.24}=0.21$ is a rough estimate for the upper limit of the correlation that should result from (a).

The fact that of the four coefficients of correlation between weaning weight and y_1 presented, only one falls well within the expected range, two are negative, and one significantly so, indicates that some path of relation other than (a) exists between the two variables. Mumford (*15*) reported that if calves' growth is limited nutritionally during a part of their growth period they will grow more rapidly when nutrition is optimum. Woodman and Evans (*20*) and Jackson (*10*) reported the same phenomenon in swine and rats, respectively. This sort of occurrence, which might be termed "compensatory growth," is a reasonable explanation for the size and sign of the observed correlations since, as discussed in the first section of this paper, growth prior to weaning is often limited by nutrition.

The coefficients of variation (C. V.'s) of y and y_1 were found to be 0.0944 and 0.0970 respectively. Thus instead of being lower, C. V. y_1 was slightly, though perhaps insignificantly, higher than C. V. y. A tentative explanation is that uniformity gained by calculating rate of gain from a standard weight is offset by what has been termed "compensatory growth." In the case of y these opposing forces would partly offset each other. Assuming this to be the correct explanation and reasoning a bit further, if compensatory growth did not occur, C. V. y would be greater than C. V. y_1. The greater the effect of compensatory growth the larger C. V. y_1 would be and, other things being equal, the smaller the difference (C. V. y–C. V. y_1) would become (in the arithmetic sense with regard to sign). At the same time the greater the effect of compensatory growth the lower becomes the correlation (r_{wy1}) between y_1 and weaning weight (again with regard to sign). Thus, if the above interpretation is correct there should be a positive correlation between (C. V. y–C. V. y_1) and r_{wy1} when they

are both calculated for several groups of pigs. Actually, a correlation of 0.61 was found between them with 16 different groups of pigs concerned.

One conclusion to be drawn from the foregoing interpretation of the statistics presented concerning growth is that rate of growth from 50 to 200 pounds is biased in favor of the light weaning pig. Another is that the bias in postweaning rate of growth calculated from a standard initial age in favor of the heavy weaning pig, reported by Smith and Donald (*17*), is not as serious as may at first appear since it is at least partly offset by the effect of compensatory growth.

The correlations between weaning weight and rate of gain are all lower for the Minn. No. 1 pigs than the corresponding correlations for the Poland Chinas. The only one of the four differences between corresponding coefficients of the two groups not statistically significant is the one involving between litter variations of weaning weight and y. A difference in the amount of compensatory growth occurring in the two kinds of swine is a possible explanation. This could result from a greater divergence in opportunity for growth up to weaning. The evidence is against this in the case of the within-litter correlations since the variance of weaning weight for the Minn. No. 1 and the Poland China pigs was practically the same within litters (table 4). The variance between litters is higher for the Minn. No. 1 pigs. Smith and Donald (*17*) suggested that a possible source of positive correlation might be permanent injury to the pig's ability to grow by some factor that caused low weaning weight. This would be exactly opposite to the situation in which lack of early growth was compensated by more rapid gains at a later date. It is possible that the Minn. No. 1 pigs are less susceptible than the Poland Chinas to permanent injury to their growth ability. They are a vigorous strain and accordingly may be better able to come through a period of slow growth and make up for it when conditions are favorable. Continuous observation of the pigs would if anything serve to reinforce one's belief in this possibility.

TABLE 4.—*Mean squares between and within litters for weaning weight, by breed and station*

[Data for 3 years combined on an intrafarrow basis]

Breed and source of variance	Degrees of freedom	Mean square	F
Poland China:			
Between litters	177	165.5	6.06**
Within litters	1,011	27.2	
Minn. No. 1:			
Between litters	63	306.6	11.4**
Within litters	422	26.9	

**Indicates $P < 0.01$ that F of this size would occur through chance.

The correlations between weaning weight and y_1 are smaller between than within litters. This is probably due to nutritional and other environmental limitations varying more between than within litters, thus giving more opportunity for the compensatory growth factor to operate.

HERITABILITY OF GROWTH DURING DIFFERENT PARTS OF THE GROWING PERIOD

The improvement of future over present generations that can be brought about through selection depends on the extent to which variations in the characteristic concerned are transmissible from parent to offspring. Heritability of weaning weight, 180-day weight, rate of gain from weaning to 200 pounds, and rate of gain from 50 to 200 pounds were, therefore, estimated in order to throw further light on their comparative values in selection for growth rate.

The method of intrasire regression described by Lush (*13*) was used. The basis for the regressions was somewhat narrower than implied by intrasire, since the data were sorted into groups of litters born in the same year and season, sired by the same male, and out of dams born in the same year and season. Genetic variance thus was measured in relation to only that part of environmental variance that appears among pigs out of sows of the same age and within farrows. There were 172 dam-litter pairs making up 38 groups as defined above. Thus regression coefficients were based on 133 degrees of freedom.

Table 5 contains the regression coefficients of offspring on dam for the items listed. None of them differs from zero by an amount large

TABLE 5.—*Coefficients for the regressions of offspring on dam for various measures of growth*

Measure of growth	Coefficient of regression	Standard error	Estimate of genetic portion of variance [1]
Weaning weight	−0.095	0.070	
180-day weight	.071	.090	0.14
Average daily gain from weaning to 200 pounds	.155	.086	.31
Average daily gain from 50 to 200 pounds	.130	.086	.26

[1] Since these estimates were calculated on an intra inbred-line basis they refer to the average genetic portion of variance within the inbred lines. Estimates of the average genetic portion of the variance within the outbred stocks from which the lines were started would be somewhat higher.

enough to be statistically significant. However, the regression coefficients for both measures of postweaning growth are larger than those for weaning weight and 180-day weight. The differences between them and the coefficient for weaning weight are statistically significant. The negative sign of the coefficient for weaning weight can hardly have any biological significance. It must have arisen from random variation.

The accuracy with which 180-day weight of offspring was predicted by different measures of the growth rate of their dams was compared by calculating the amount of variation in 180-day weight of offspring associated with variation in the various measures of dam's growth. The sum of squares for 180-day weight of offspring was 64,972. Regression on 180-day weight of dam accounted for 301 of this total. Comparable figures for regression on rate of gain from weaning to 200 pounds and on rate of gain from 50 to 200 pounds of the dam were 898 and 1,714 respectively. The differences among these 3 figures were not statistically significant. It is, however, entirely conceivable that postweaning growth rate of dams actually predicts 180-day weight of offspring more accurately than does 180-day weight of dams.

DISCUSSION

The evidence presented indicates that the pigs studied did not receive optimum nutrition during at least a part of the suckling period. This, together with data reported by Donald (*5*, *6*, *7*) and Bonsma and Oosthuizen (*1*) on the inequality of nutritional opportunity both within and between litters, leads to the conclusion that weaning weight can be but a poor criterion of inherited ability to grow. Bywaters' (*3*) estimate of the proportion of variance in weaning weight that is additively genetic (4 percent) and the regression of offspring on dam for weaning weight herein reported support this conclusion. Weight at weaning is a part of 180-day weight. The correlations between weaning weight and rate of gain from weaning on for the Poland China swine (table 3) are larger than would result from genetic causes alone. This means that heavy weaning pigs have gained more in the period from 56 days on as a result of being heavier at the beginning of that period. Thus weaning weight affects 180-day weight in two ways, (1) it is itself a part of 180-day weight and (2) it is directly responsible for a part of the variation in weight gained in the period from 56 to 180 days. It is apparent that if postweaning growth is more heritable than weaning weight, it must also be more heritable than 180-day weight. The data indicate that postweaning growth rate is more heritable than weaning weight and, as expected, 180-day weight falls between them in this respect. However, the true difference between the heritability of postweaning growth and 180-day weight is probably not as large as here estimated since the true regression for weaning weight must actually be positive though small.

It could be logically argued that 180-day weight or rate of gain from birth to market weight should be used for selection since they measure what is actually concerned in the production of market hogs. On the other hand, there is a strong possibility that postweaning growth rate is a better measure of ability to grow prior to weaning than weaning weight itself. This could be true if the genes affecting growth have somewhat the same relative effect before and after weaning. It would not necessarily be true if part of the genes for growth were specific for short periods of the animal's life. There is evidence for the former situation in the fact that 180-day weight of offspring is predicted more accurately in the data studied by postweaning growth rate of dam than by 180-day weight of dam.

The correlations between weaning weight and the two measures of postweaning growth have been discussed with regard to the channels of relationship which contribute to them. The conclusion appears justified that rate of gain from 50 to 200 pounds is biased in favor of light weaning pigs while rate of gain from weaning to 200 pounds probably tends to favor heavy weaners. The latter would be more useful in selection because it is desirable to favor heavy weaning weights if it can be done without sacrificing something else. Even though weaning weight were entirely independent of the pig's genotype it should get some emphasis as a means of selecting for heavy milking qualities in the dam.

In these data 180-day weight of offspring was not predicted as well by dams' rate of gain from weaning to 200 pounds as by dams' rate of gain from 50 to 200 pounds. This probably resulted from the fact

that there was a negative correlation between the weaning weight of dam and offspring, which it is to be remembered was probably fortuitous. On the average this correlation should be positive though small. In that case rate of gain from weaning to 200 pounds would probably predict 180-day weight of offspring slightly more accurately than rate of gain from 50 to 200 pounds.

CONCLUSIONS

Nutrition of pigs prior to weaning is not optimum, nor is it equal for all individuals. If this situation could be corrected by a suitable method of supplementary feeding during the suckling period, the heritability of weaning and 180-day weights should be increased. The evidence indicates that with present feeding methods measures of postweaning growth will be more useful in selection for growth rate in swine than measures of growth rate embracing the entire period from birth to market weight. It can be tentatively concluded that since the two postweaning growth rates described appear to be equally heritable, rate of gain from weaning to 200 pounds should give better results because it favors the heavy weaning pig.

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PRODUCTION OF ACTIVE AND INACTIVE CATALASE BY PROTEUS VULGARIS [1]

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INTRODUCTION

Considerable general information on the production of catalase by micro-organisms is available. As a rule, the reports discuss the production of catalase by different species of bacteria and fungi, without giving much specific information regarding its production under various conditions and at different stages of growth.

The existence of catalase in an inactive state has long been recognized. Batelli and Stern (*4*) [3] inactivated the catalase in preparations from liver by shaking them in air; reactivation was brought about by the subsequent addition of small quantities of alcohol. Balls and Hale (*3*) found that the catalase activity of extract from hog liver could be greatly increased by the addition of substances containing S–S groups, such as cystine and oxidized glutathione. However, not all liver preparations reacted in this way. An apparent increase in catalase could be produced by the same means in extracts of various grains and in cultures of several micro-organisms.

The mode of action of these activators is not evident. Their effect may be due to surface action, to the removal of inhibiting substances, or to a change in the enzyme itself, as the completion of some step in the chain of reactions leading to the decomposition of hydrogen peroxide. The research of Keilin and Hartree (*26*, *27*) indicates that the active catalyst is an oxidized form. Oxidants such as S–S compounds may conceivably alter an equilibrium in the crude catalase preparations in favor of the oxidized form.

Recently Stern and Wyckoff (*46*, *47*) ultracentrifuged catalase extractions from horse liver and obtained an extremely active fraction that had k values from 11,700 to 161,000. Sumner and Dounce (*48*, *49*, *50*) extracted beef liver with dilute dioxane and then precipitated the catalase with more concentrated dioxane, finally obtaining a crystalline fraction that they believed to be pure catalase. This fraction contained 0.1 percent of Fe, had an isoelectric point of 5.7, and according to Sumner and Gralén (*51*, *52*), had a molecular weight of 248,000.

Balls and Hale[4] found that a trichloracetic acid extract of lean heart muscle, made according to their own technique, activated fresh preparations of catalase from one to five times. When used with aqueous suspensions of *Proteus vulgaris* this extract produced activations of about the same order. The authors found that cholesterinized antigen, made according to the technique reported by Kolmer (*30*), activates catalase.

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[3] Italic numbers in parentheses refer to Literature Cited, p. 401.
[4] Unpublished results.

The investigations reported here were conducted with a single culture of *Proteus vulgaris* to determine the quantitative variations that occur (1) in the active and activable catalase during a fairly complete growth cycle, and (2) in the effects of various activators on activable catalase when time, temperature, and oxygen concentration are varied and when cell death occurs.

REVIEW OF LITERATURE

The power of bacteria to decompose hydrogen peroxide was first observed in 1893 and was independently reported by Beijerinck (*5*) and by Gottstein (*12*). Loew (*33*) in 1901 announced the discovery of a new enzyme, which destroys hydrogen peroxide, with the liberation of free oxygen. This enzyme, which is of general occurrence in plants, he named catalase.

This discovery stimulated other investigators in the field of microbiology to study catalase production by bacteria, yeasts, and fungi. Catalase is now recognized as one of the most widespread of all known ferments, and its behavior has been investigated in great detail. In 1903 Bach and Chodat (*1*) reported its presence in *Sterigmatocystis nigra*. In the same year Löwenstein (*34*) reported that he was unable to find catalase in cultures of the tetanus organism. In 1904 and 1905 Issajew (*17*, *18*) found in yeast a catalase that he could extract with glycerol and precipitate with alcohol. Orla-Jensen in 1907 reached the conclusion that catalase is not commonly found in the anaerobic butyric acid bacteria (*40*). This was substantiated by Virtanen in 1925, when he reported also that catalase was not present in the true lactic acid or propionic acid bacteria (*54*).

In 1907 Rywosch and Rywosch (*42*) made a study of the production of oxygen from hydrogen peroxide by bacteria, yeasts, and fungi. They found that the quantity ranged from as much as 7.0 cc. per mg. produced by a species of *Sarcina* to none by species of *Clostridium*. The next year, 1908, Jorns studied 90 strains of bacteria and actinomycetes and arranged them according to the quantity of catalase produced (*25*). He found that the greatest quantity was produced by *Bacterium prodigiosum*. In 1909 Pringsheim determined the quantity of catalase produced by a number of different fungi (*41*).

In 1910 Dox found that many species of *Penicillium* and *Aspergillus* contain catalase in varying quantities (*9*). It is first present in the intracellular form, and then as the cultures become older it diffuses into the medium.

In 1918 and 1919 Jacoby studied the catalase extracted from *Proteus vulgaris* by treatment with ammonium sulfate and with methyl alcohol (*21*, *22*, *23*). When he tested the decomposition of hydrogen peroxide by the catalase extract at different pH and hydrogen peroxide values he found that although in some cases the k value remained constant during the course of hydrogen peroxide decomposition, in other cases it increased and in still others it decreased.

He later (*24*) measured the composition of hydrogen peroxide by 64 cultures of normal strains of *Proteus vulgaris* and by 7 cultures of the x-strain. He found that whereas the normal strains decomposed an average of 81.6 percent, only 5 cultures falling below 50 percent, the 7 cultures of the x-strain averaged only 31.5 percent, 6 of these falling below 50 percent. In 1923 Hagihara (*13*) determined the

content of nitrogen and of ash and the dry weight of purified catalase from the *P. vulgaris* cultures used by Jacoby.

In 1923 Callow (*8*) observed that the anaerobes and the streptococci alone are free from catalase, while McLeod and Gordon (*36*) reported that it is present in the facultative anaerobes but absent in the obligate. The same authors made the interesting observation that the pneumococci inhibit their own growth by the formation of hydrogen peroxide. In 1923 Ohtsubo (*39*), in a study of catalase production by 164 strains of 11 species of bacteria, observed variable results from the same species. In 1925, Burnet (*7*) found catalase in four species of bacteria but failed to find any in *Streptococcus hemolyticus*. Kluyver (*29*) in 1924 stated that no catalase is produced by *Clostridium putrificum*. In the same year, Stapp (*45*) reported finding catalase in all bacteria except the streptococci, more being produced by bacteria cultivated under aerobic conditions than by those grown anaerobically. In 1925 Virtanen and Karström reported that 15 different species of bacteria investigated produced catalase (*55*). They found that *Bacterium pyocyaneum* and *Bacterium lactic amari* produced more catalase when cultivated anaerobically than under aerobic conditions.

In 1926 Hooft reported that all species of acetic acid bacteria, with the exception of *Acetobacter peroxydans*, produce catalase (*16*). Kirchner and Nagell in 1926 found that strains of gonococci contained about 3 times as much catalase as straphylococci, which in turn contained about 10 times as much as *Bacterium coli* (*28*). Sherman (*44*) in 1926 and Van Niel (*38*) in 1928 noted the presence of large quantities of catalase in propionic acid bacteria.

According to a report of Virtanen and Winter (*56*) published in 1928, the production of catalase by *Bacterium coli* is independent of the pH of the medium and the low catalase content observed in media containing glucose may be attributed to the production of strong acid, which causes the death of many of the cells. *B. coli* grown in strongly oxygenated media produced more catalase than when grown on oxygen-free media.

In 1928 Fernández and Garméndia (*11*) stated that the production of catalase by *Bacterium coli* increases or decreases according to the media. They concluded that a classification of strains on the basis of catalase production is impossible.

Itano and Arakawa (*19*) found in 1928 that a culture of *Clostridium thermocellum* and a thermophilic cellulose-fermenting bacterium isolated by Itano had maximum catalase activity when the culture was 72 hours old. The velocity of reaction decreased on standing, apparently because of the destruction of the enzyme.

Iwanoff, Dodonowa, and Tschastuchin reported in 1930 that the fruiting bodies of the mushrooms *Psalliota campestris*, *Lepiota procera*, and *Coprinus radiatus* contain the enzymes maltase, glycogenase, amylase, protease, and catalase (*20*). Maximum quantities were found at full maturation.

In 1931 Wolfson reported that certain dyes had an inhibiting influence on the formation of catalase by bacteria (*57*). The effect was more pronounced on the Gram-negative types than on the Gram-positive.

The catalase activity of *Azotobacter* is relatively insensible to acidity and high temperatures, according to the report of Burk, Horner, and

Lineweaver (*6*) published in 1932. The optimum for catalase production was found to be a neutral medium.

In 1933 Matsuyama found that bubbling air through cultures of yeast during the first stages of growth increased the catalase activity (*37*).

Striking variations in catalase in representatives of the genus *Propionibacterium* was noted by Hitchner (*15*) in 1934. Talce-Niedra reported in 1936 that certain strains of the *Streptoccocus lactis* group produce catalase (*53*). When these were cultured anaerobically, however, the catalase production was low.

In 1937 Edwards and Newton reported that when peptone was the sole source of nitrogen in the medium used to grow *Rhizoctonia solani*, catalase was high, whereas when gelatin, potassium nitrate, ammonium sulfate, or urea was used the catalase activity was low (*10*). In 1938 Kirshnan reported that he was unable to detect catalase in cultures of *Nitrosomonas* (*32*).

Hayasida (*14*) in 1938 found very strong catalase activity in *Fusarium*. He states that this genus contains enzymic systems which affect true alcoholic fermentations of hexoses, being similar to yeasts in that respect.

In 1940 Van Schouwenburg (*43*) found that the catalase content of all species of luminous bacteria studied produced catalase. He determined catalase production by measuring the quantity of oxygen produced from hydrogen peroxide in the Warburg apparatus.

This review of the literature seems to indicate a conflict in the findings of the different investigators. The conflict may be more apparent than real, however, as environmental conditions also appear to have considerable influence on the production of catalase by micro-organisms. Therefore, in order to classify organisms on the basis of catalase production, it would be necessary to determine the effect of the environment on each species or group of species. Nevertheless McLeod and Gordon (*35*) in 1923 proposed to divide bacteria into the following four groups:

1. The anaerobes. These are devoid of catalase, extremely sensitive to H_2O_2, and potentially peroxide producers.
2. Peroxide producers. These are devoid of catalase and only moderately sensitive to H_2O_2. In this group are the pneumococci, many types of streptococci, the lactic acid bacteria, and some sarcina.
3. Nonperoxide producers. These are devoid of catalase. This group includes certain streptococci, dysentery baccilli (Shiga type), and some hemoglobinophilic bacteria.
4. Catalase producers. This group includes the majority of the bacteria growing aerobically and also the facultative anaerobes.

PROCEDURE

BACTERIOLOGICAL METHODS

A culture of *Proteus vulgaris* that had shown remarkable ability to produce both active and activable catalase was used throughout this investigation.

Since preliminary work had indicated that the quantity of catalase produced on agar slants was greater than that produced in nutrient-broth cultures, agar slants were employed. The greater production on agar slants may be attributed to the more favorable oxidizing conditions on the slants.

The nutrient agar used as culture medium was made as follows:

Beef extract (Liebig)	5 gm.
Sodium chloride	5 gm.
Peptone (Armour)	10 gm.
Distilled water	1,000 ml.
Agar	15 gm.

The ingredients were boiled for 20 minutes and made up to the original volume with distilled water; 1.5 percent of granular bacto-agar (Difco) was then added, and the preparation was steamed until the agar was melted. The medium was then adjusted to pH 7.3 colorimetrically and filtered through a glycerin filter paper. It was tubed in 10-ml. aliquots by means of a volumetric pipette. The tubes were plugged and sterilized at 15 pounds pressure for 40 minutes. The final pH after sterilization was 7.0, the optimum for catalase reactions.

For the agar slants, 20- by 150-mm. culture tubes were selected on the basis of uniformity of inside diameter to insure the same growth surface in each tube. All tubes were slanted at the same angle. In addition to the agar slants, two lots of water blanks consisting of 10 ml. each of buffer solution (0.2 molar phosphate, pH 7.0) were made and sterilized. A second series of blanks consisting of 9 ml. each of distilled water was prepared with each batch of the medium.

A 24-hour agar slant of the organism (*Proteus vulgaris*) was used for seeding the slants. The growth on this slant was washed off and suspended in 10 ml. of the sterile buffer solution. One milliliter of the buffer suspension was mixed with a 99-ml. sterile water blank, and the seeding for the entire experiment was made from this dilution. Seeding was accomplished by dropping 0.1 ml. of this diluted buffer suspension on the upper end of the slant and allowing it to flow down over the surface. In order to minimize the possibility of a lag or death phase because of temperature differences, all tubes of agar and water blanks were kept in the incubator for 18 hours before inoculation. All bacteriological plate counts were made after 48 hours' incubation at 37.5° C.

A sufficient number of agar slants were seeded to provide two for each determination from the initial inoculation up to and including the final determination. The time interval between determinations is given in the data for each experiment.

Determinations were made at the time of the initial inoculation and then every hour for 24 hours, then every 4 hours up to 40 hours, and then at 48, 52, 72, 96, and 120 hours, respectively. The same determinations were made for active catalase and catalase after activation with calf-heart extract and with Kolmer antigen.

In addition to the growth slants, three unslanted tubes were provided for plate counts. At the time of each analysis the cultures for that particular analysis were washed from the two agar surfaces and suspended together in 10 ml. of buffer solution. One ml. of this suspension was then transferred to a 9-ml. sterile water blank, and this in turn was further diluted for the plate count. Plates were poured in triplicate, and 10 ml. of agar was added to each plate containing the dilution of organisms, thus assuring identical conditions in each plate. The catalase determinations were made from the remaining 9 ml. of buffer suspension.

CATALASE DETERMINATIONS

The procedure of Balls and Hale (*2, 3*) was used for the determination of catalase. The technique was as follows:

The surface growth of organisms on two tubes was washed off and suspended in 10 ml. of buffer solution. Two milliliters of this suspension was mixed with 1 ml. of buffer solution and 1 ml. of ice-chilled distilled water (previously boiled to expel air). Two milliliters of this mixture, equivalent to one-fifth of the enzyme material on one slant, was then added to the hydrogen peroxide for determination of the active catalase.[5] The activable catalase was determined by incubating 2 ml. of the same buffer mixture with 1 ml. of enzyme activator and 1 ml. of phosphate buffer (pH 7.0) for 10 minutes before mixing with the hydrogen peroxide for analysis.

The quantity of catalase used was sufficient to decompose at least half the peroxide in 5 to 7 minutes. It was pipetted into a stoppered cylinder containing 1 ml. of 0.2 normal hydrogen peroxide,[6] 4–5 ml. of 0.2 molar phosphate buffer (pH 7.0), and approximately 1 gm. of freshly dissolved dextrose in sufficient water to make, with the added catalase, a total volume of 50 ml. The cylinder and contents were previously cooled in an ice bath. After addition of the catalase, the cylinder was shaken and replaced in the ice. As soon as convenient a 10-ml. aliquot was pipetted (with a rapidly flowing pipette) into a flask containing 20 ml. of 2 normal sulfuric acid and 5 drops of a saturated water solution of molybdic acid. Ten milliliters of 10-percent potassium iodide was added at once, and the contents of the flask were allowed to stand about 4 minutes, after which the liberated iodine was titrated in the usual manner with 0.01 normal thiosulfate.

Another 10-ml. aliquot was taken in about 2 minutes and a third in about 5 minutes. The time is rather at the discretion of the operator, who must know something of the strength of his preparation. The moment when the delivery pipette is half empty is noted on a stop watch in each case. The first titration used at the start of the experiment gives the initial concentration of peroxide (a). The subsequent titrations give the peroxide remaining ($a-x$) at the time (t) of observation. Substitution in the usual formula, $1/t \log a/a-x=k$, gives the monomolecular constant, which varies directly with the quantity of the enzyme and can be used as a unit for measuring the enzyme.

Small variations in the hydrogen peroxide concentration do not affect the results, and since the value of k depends on a ratio, the exact standardization of thiosulfate and peroxide is unnecessary, but the reagents should be of approximately the specified concentrations. Excusable variations from the average value of k by this method would seem to be about 10 percent.

[5] It was found that the most consistent results were obtained if the 2-ml. sample was taken as soon as the mixture was complete.

[6] The hydrogen peroxide used should be free of the preservatives ordinarily added to stabilize it. The writers prepared their own. Exactly 7.80 gm. of the best sodium peroxide obtainable was added, little by little, with stirring, to 1 liter of water at 0°–2° containing 54.4 gm. of the purest potassium acid phosphate (KH_2PO_4). Practically no evolution of oxygen occurred. After the sodium peroxide is dissolved, the solution should have a pH value of 7.0–7.1. It contains 0.2 normal hydrogen peroxide and 0.2 molar phosphate buffer. At a low temperature this stock solution keeps fairly well.

RESULTS

COMPARISON OF POPULATION AND CATALASE ACTIVITY AT VARIOUS TIME INTERVALS

The curves obtained by plotting the number of bacteria per milliliter against the time of incubation of the organism and the k values against the age of the culture are shown in figure 1.

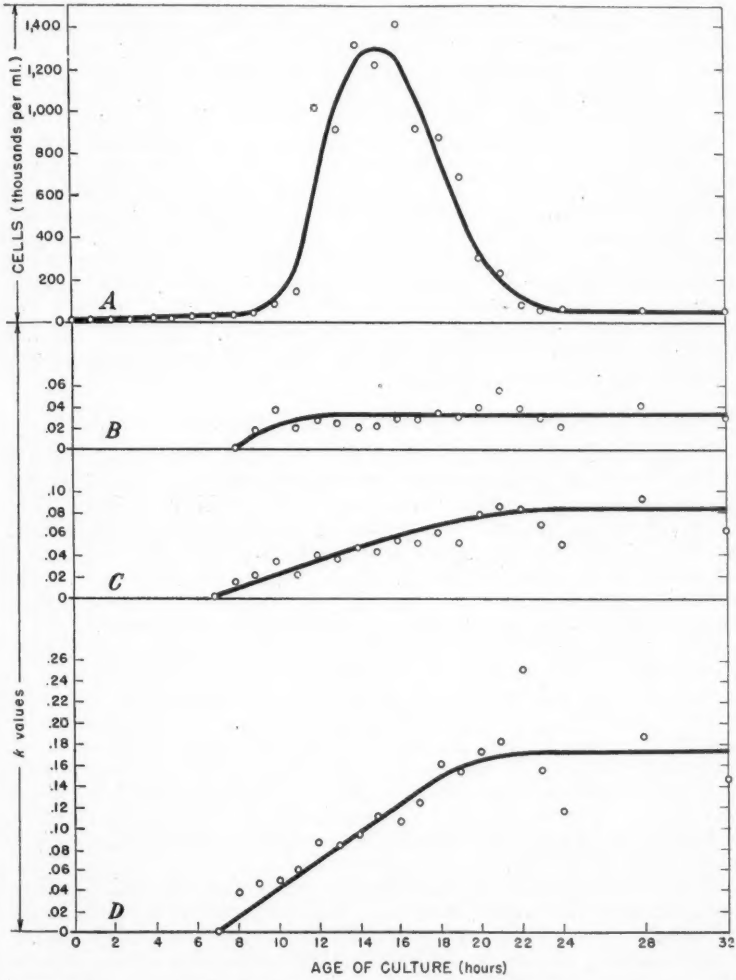

FIGURE 1.—*A*, number of viable cells of *Proteus vulgaris; B*, catalase produced with no treatment; *C*, catalase produced after treatment with calf-heart extract; *D*, catalase produced after treatment with Kolmer's antigen.

The bacterial population curve in general follows a normal course in conformity with usual theoretical growth curves. The peak was reached at 16 hours, after which there was a rapid decrease to 70,000 per milliliter at 22 hours, then a more gradual decrease to 11,000 at 120 hours.

The quantity of active catalase was practically negligible until after 9 hours; that is, during the lag phase of the growth of the organism, no appreciable quantity of active catalase was present. The quantity of active catalase then increased gradually until a maximum was reached at 21 hours, which was several hours after the peak was reached in the bacterial population. After this the quantity fluctuated more or less irregularly, being only slightly lower at 120 hours. In general, the activated catalase, both that activated by treatment with calf-heart extract and with Kolmer antigen, followed the same course as the active catalase, except that the quantity of the activated form was considerably greater. The average k value for active catalase was 0.02856, for catalase activated by calf-heart extract it was 0.05831, and for catalase activated by Kolmer antigen it was 0.12572. If it is assumed that the activator did not affect the active catalase present, then the treatment with calf-heart extract activated as much catalase as was originally active. The calculated ratio was 1:1.04. The Kolmer antigen was considerably more effective. The ratio in this case was 1:3.40; that is, nearly 3½ times as much catalase was activated as was originally active.

EFFECT OF OXYGEN ON NUMBER OF VIABLE CELLS AND ON CATALASE

It seems probable that the oxygen in the culture tubes was depleted during the period of most active growth and that the lack of oxygen then caused a rapid decrease in the number of viable organisms. The curve for active catalase (fig. 1) shows a steady increase from 9 to 21 hours, indicating a lack of correspondence between the greatest rate of growth of the organism and maximum production of catalase. The following experiment was conducted to determine the effect of oxygen on the number of bacteria and on the production of catalase.

The cultures of *Proteus vulgaris* were first incubated on U-tube agar slants. The tubes were kept under optimum conditions for 16 hours and then subjected to the respective percentages of oxygen for 2 hours. Each arm of the tube had a side outlet. An oxygen-nitrogen gas mixture was allowed to flow into one side arm, passed over the surface growth on the agar slant, and then out through the opposite side arm. Oxygen concentrations of 0, 10, 25, 50, 75, and 100 percent were used. The total bacterial population, quantity of active catalase, and quantity of catalase present after activation with Kolmer antigen then were determined.

The k values were calculated by the usual formula, and k_1 values were calculated by multiplying the k value by the factor 10^8 and then dividing by the number of viable cells in the bacterial population.

The results, shown in table 1, indicate that the production of catalase, active and activable, by *Proteus vulgaris* was stimulated by oxygen and that there was an increase in the quantity produced up to and including a concentration of 75 percent of oxygen. When a concentration of 100 percent of oxygen was used, there was a marked decrease. The calculated k_1 values, that is, the catalase produced

per cell, show the marked increase up to 75 percent of oxygen and then the rapid decrease at 100 percent.

TABLE 1.—*Effect of free oxygen on the production of catalase by Proteus vulgaris*

Oxygen (percent)	Active catalase	Activated catalase	Ratio of active to activated catalase	Active catalase	Activated catalase
	k value	*k value*		k_1 *value*	k_1 *value*
0	0.0141	0.0248	1:0.76	2.01	3.54
10	.0355	.1363	1:2.84	2.53	9.72
25	.0229	.1109	1:3.84	3.81	18.40
50	.0442	.1865	1:3.21	4.91	20.70
75	.0333	.1654	1:3.96	8.32	41.30
100	.0091	.0338	1:2.71	2.27	8.45

The ratio of active catalase to activable catalase shows that between 10 and 75 percent of oxygen the increases in the two forms of catalase approximately parallel each other, that is, the ratio varies only 15 percent, whereas both 0-percent and 100-percent concentrations of oxygen seem to be detrimental to catalase production. The chief effect of increasing the concentration of oxygen from 10 to 75 percent was on the number of viable cells. This is illustrated by the consistent change in k values, which is dependent on the catalase concentration and, therefore, on the number of living cells.

EFFECT OF TEMPERATURE ON THE RATE OF ACTIVATION

Activation of the inactive catalase at 0° and at 37.5° C. was compared. Kolmer's antigen was used as the activator. Rates were determined at the start and after 1, 5, 7, 10, 20, 30, and 60 minutes. The k values were calculated at the end of each of these time intervals. The results are given in table 2.

TABLE 2.—*Effect of temperature on rate of activation*

Time (minutes)	Activation at—		Time (minutes)	Activation at—	
	0° C.	37.5° C.		0° C.	37.5° C.
	k values	*k values*		*k values*	*k values*
0	0.0373	0.0324	10	.....	.0352
1	.0396	.0734	20	.....	.0262
5	.0382	.0892	30	.0354	.....
7	.0306	.0647	60	.0490	.....

From the figures in table 2 two points are evident: (1) At 0° C. enzyme activity remained approximately constant; and (2) at 37.5° it increased rapidly, reaching the highest rate at 5 minutes and then declining until at 20 minutes it was lower than the initial rate, thus indicating destruction of the catalase at the higher temperature.

EFFECT OF DESTRUCTION OF THE CELL ON CATALASE PRODUCTION AND ACTIVATION

The difference between activability at 0° and at 37.5° C. suggests that activation may be closely connected with the metabolic activity of the cell. This indication is further supported by the following experiments.

A suspension of *Proteus vulgaris* was made from an agar slant as previously described. The number of viable cells in the 10-ml. buffer suspension was found in the usual manner, and the total active and activable catalase was determined. This required 3 ml. of the suspension. The remaining 7 ml. was then centrifuged at high speed for 15 minutes, and the supernatant liquor discarded. The cellular precipitate was resuspended in acetone at 37.5° C. and stirred for 15 minutes. It then was centrifuged to remove the acetone. The acetone was discarded, and the cell precipitate suspended in 7 ml. of buffer water. The number of viable cells and the total quantities of active and activated catalase were determined. The results were:

Sample 1.—Viable cells per milliliter before treatment, 25,000,000; after treatment, 310,000. k value of the active catalase before treatment, 0.0113; after treatment, 0.0133. k value of the activated catalase before treatment, 0.0564; after treatment, 0.0117.

Sample 2.—Viable cells per milliliter before treatment, 40,000,000; after treatment, 500,000. k value of the active catalase before treatment, 0.0433; after treatment 0.0400. k value of the activated catalase before treatment, 0.1092; after treatment 0.0495.

The results show that the number of viable cells markedly decreased. The quantity of active catalase was not affected by the treatment, but the activable catalase was either destroyed or the treatment used for activation was not effective in the absence of viable cells. This seems to be evidence that to be activated activable catalase must be in the presence of living cellular material.

ABSENCE OF CELL-FREE CATALASE, EITHER ACTIVE OR ACTIVABLE

A suspension of cells containing catalase that showed good activation was mixed with the proper quantity of activator and centrifuged at a high rate for 10 minutes (the usual period of activation). The supernatant liquor was decanted, and the precipitate thrown down by centrifuging was resuspended in a buffer solution. The active and the activated catalase in the supernatant liquor and the activated catalase in the resuspended precipitate were determined.

The results were: Before centrifuging, the k value of the active catalase was 0.0373; activated catalase, 0.0931. After centrifuging, the k value of the active catalase of the supernatant liquor was 0, as was also that of the activated catalase; the k value of the resuspended precipitate catalase, activated, was 0.0909.

The results show clearly that neither the active nor the activated catalase was elaborated into the medium. This catalase, therefore, is in the "desmo" class.

CONCLUSIONS

From data presented here the following conclusions seem warranted:

Proteus vulgaris produces catalase during all its growth, except in the lag phase.

Proteus vulgaris produces two forms of catalase, active and activable. The activable form can be changed to the active form by an activator. Kolmer's cholesterinized antigen, used as an activator, produced approximately a fourfold increase in active catalase.

When the culture was treated with oxygen in concentrations up to 100 percent, concentrations of oxygen up to 75 percent brought about

a progressive increase in catalase production. This may be attributed to the stimulating effect of oxygen on the number of viable cells.

A study of activation at 0° C. as compared with that at 37.5° showed that no activation occurred at 0° up to 60 minutes, whereas marked activation occurred at 37.5° in 5 minutes. That there is a correlation between bacterial activity and rate of activation is offered as an explanation for this. Instability of the enzyme at the higher temperature was indicated by the decrease in activity after 8 minutes.

The activable form of catalase cannot be extracted by acetone and therefore may be regarded as an integral part of the living cell, that is, a desmo enzyme.

When the viable cells were destroyed, the activable catalase was either destroyed or could not be activated in the absence of viable cells.

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